CHEMICAL CONSTITUENTS OF SOME PLANTS OF BUNDELKHAND REGION

THESIS

Submitted to the Bundelkhand University, Jhansi

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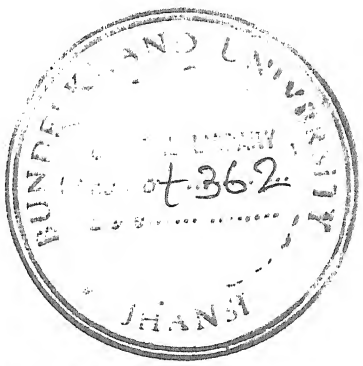
(Faculty of Science)

By

Km. Raka Jain



Organic Chemistry Laboratory

Indian Grassland and Fodder Research Institute

JHANSI (U. P.)

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C E R T I F I C A T E

This is to certify that the thesis entitled, "Chemical Constituents Of Some Plants Of Bundelkhand Region," submitted for the degree of Doctor of Philosophy of Bundelkhand University, Jhansi (U.P.), is a record of bonafide research work, carried out by Km. Raka Jain, M.Sc. Senior Research Fellow, C.S.I.R., under my guidance and supervision.

No part of the thesis has been submitted for any other degree or diploma. All the assistance and help received during the course of investigation have been duly acknowledged.

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A C K N O W L E D G E M E N T

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Raka Jain
(RAKA JAIN)

C O N T E N T S

PREFACE

For aeons man has relied upon natural products as a prime source of food, medicine and materials for shelter and construction. The amazing diversity and complexity in chemical nature of plant products together with the tiresome operations involved in the isolation of pure compounds from very complex mixtures of closely related and unrelated compounds, often present in insignificant amounts, had been a major obstacle in the progress of plant chemistry. Despite all these difficulties, considerable amount of work recently done on plant products, has resulted in a series of achievements, in isolation, identification and total synthesis of a variety of complex natural products, such as, alkaloids, carbohydrates, terpenes, flavonoids, steroids, colouring matters, fats and related compounds, the chemistry of which had defied elucidation for a considerable time.

The tremendous sophistication introduced during the last few years in techniques of isolation, identification and structural elucidation now makes it possible to know the structures of new biologically active natural products in a relatively short time. Many novel structural types possessing biological activity have been obtained in recent years from the plants which have greatly increased the potential leads coming from these sources. The chemistry of natural products is becoming more and more closely involved with biology and life

processes particularly during the last few decades and the area of biosynthesis has seen unparalleled advances.

Efforts in the past have resulted in the discovery of many drugs of plant origin. One of the most interesting achievements of 19th and 20th century chemists was to isolate the active principle from a number of vegetable drugs, such as morphine[1] from Papaver somniferum Linn. (Papaveraceae), quinine[2] from Chinchona officinalis Linn. (Rubiaceae), resperine[3] from Rauwolfia serpentina Benth. (Apocyanaceae), α-santonin[4] from Artemisia maritima Linn. (Compositae), etc. Panax ginseng[5] C.A.Mey (Arialiaceae) is the most described saponin containing plant. Its roots under the name 'Ginseng' are commonly used in Western and Chinese medicine. Numerous saponins, named ginsenosides, have been isolated and some of them show the antifatigue activity of the original plant extract.

Glycosides are the most widely distributed compounds in the plant kingdom. The group of glycosides[6] like sinigrin from Brassica nigra Koch. (Cruciferae) and sinalbin from Sinapis alba L. (Cruciferae) yield the essential oils containing sulphur compounds which are irritant in their action and are harmful. Glycosides having direct action on the heart of peripheral circulation are classified as cardiac glycosides. Those having direct action on animals are digitoxin[7] from Digitalis lanata Ehrh. (Scrophulariaceae), strophanthin[8] from Strophanthus kombe Oliv. (Apocyanaceae) and thevetin[9]

from Thevetia nerifolia Juss. (Apocyanaceae), etc. Cyanogenetic glycosides are of general occurrence in a number of grasses belonging to the genera Graminae and Leguminosae which provide valuable fodder and feed to the livestock. They are of economic and toxicological interest.

The flavones are an important group of pigments which occur naturally and are very widely distributed.[10] Recently some flavonoids have been claimed to possess interesting biological activities. Rutin, a well known glycoside originally derived from Ruta graveolens Linn. (Rutaceae) is now being increasingly employed in the treatment of the capillary fragility.[11] It is also used in the treatment of the after effects of exposure to atomic radiations.

Koike,[12] working on the diuretic action of flavonoids, showed that the potency was increased with the number of hydroxyl groups. In addition, the flavone glycosides quercitrin and myricitrin were also found to have diuretic action.[13] Clark and Geissman[14] showed the potentiation of epinephrine effect by certain flavonoid compounds and the relation of their structure with activity. The disorders caused by nutritional deficiency and stress in laboratory animals were prevented or minimised by the addition of queretin or rutin in the vitamin deficient diet.[15] Abrose et al[16] have reported the beneficial effects of quercitrin in frostbite. Flavonoids are reported to be cardiac

stimulants and vaso constrictors. They seem to be absorbed in the intestinal canal and excreted unchanged in the urine and bile. Sokoray and Czimmer[17] found that quercitrin caused fall in blood pressure and damaged the kidney and mesenteric vessels. The effect of flavonoids on such clinical conditions as hypertension, diabetes, rheumatic fever, arthritis and pregnancy have also been studied.[18] Chrysin and Galangin were found considerably toxic to the insects while the flavones with more hydroxyl groups were only having mild action.[19-21] In contrast the methoxy compounds were far more toxic than the hydroxy flavones.[22-23] However, these flavonoid compounds are virtually nontoxic in higher animals.[24] The flavonoids and related compounds are found to exhibit photodynamic activity as shown by karanj oil from Pongamia glabra Vent. (Leguminosae) containing the furano flavone, karanjin.[25] The occurrence of chrysoeriol[26] is of biogenetic interest and its presence is attributed to the seasonal variation in the biosynthesis of flavonoids as reported earlier[27] in the case of Pyrus species Linn. (Rosaceae). The flavone constituents can be of much greater significance in aiding the differentiation of species which are otherwise morphologically and anatomically difficult to distinguish. The flavonoids which are comparatively easy to demonstrate can act as indicators.

The sterols are neutral and comparatively stable substances, which occur partly in free condition and partly esterified with higher fatty acids. They are regular constituents of animal and plant fats. Sitosterols are the most abundent and widely distributed of plant sterols but they occur as very complex mixtures. β -sitosterol is the most common amongst the sitosterols occuring in plants and has been isolated in more or less pure form from a number of sources like cotton seed oil[28], Calycanthus oil[29], wheat germ oil[30] and crepe rubber[31] besides a number of other plants.

Free sugars and cyclitols are common constituents in most of the plants. It has been reported that cyclitols like meso-inositol, pinitol possess growth promoting activity[32]. Long chain alcohols[33] like triacontanol were also found to increase percent dry weight and percentage nitrogen (dry weight basis of leaves) in sweet potatoes.

In addition, the knowledge of chemical constituents of plants has been of considerable value in revealing new sources of economic material (examples, perfumes, oils, gums, tannins, dyes, etc.) or in providing raw materials for the synthesis of complex molecules of potential interest to drug industry.

There are numerous reasons, why plant chemists investigate plants for their constituents. Firstly, one can consider that purely scientific curiosity has motivated some investigations which may possibly have been undertaken

because the plant had not previously been investigated. More common plants have been investigated for specific classes of constituents, such as terpenes, alkaloids or flavonoids or they may have been examined as part of a chemotaxonomic study in which relationships between plants are assessed on the basis of chemical constituents. Whatever be the reasons for examining the plant initially, it may follow that isolated constituents are tested for biological activity although this is not, always the case.

Another important reason for the chemical investigation of plants is that the same plant growing in different parts of the country may synthesise different constituents in varying proportions which could obviously have a significant biological activity.

Such investigations have other advantages as well and are likely to lead not only to the discovery of new biologically active compounds and products of economic importance but also the uncovering of new types of biologically active chemical structure which surpass the wildest imagination of synthetic chemists and these molecules can serve as templates for the development of new drugs.

India has been endowed with a rich and varied floral wealth due to extreme climatic and geographical variations available in the country. In temperate countries the maintenance of an adequate proportion of legumes plays an important part in the development of good pastures. The

advantages to be gained by growing grasses and legumes in association are three fold; firstly legumes add nitrogen to the soil, secondly they supply a more balanced food, thirdly they increase the yield and nutritive value of grasses growing in association. For these reasons, legumes appear to be a very desirable component of tropical grasslands as well. Though a number of species have been recorded as occuring in Indian Grasslands, unfortunately none of them occur naturally in such large numbers as to give the advantages mentioned above. Usually the grasses constitue 99% of species of forage value on our grassland. It is therefore, necessary to find ways and means of increasing the proportion of forage legumes on our grassland.

Bundelkhand region is quite rich in its unexplored floral wealth. A wide variety of plants, bushes and trees are available which have not been studied for their chemical constituents, biological activity and for the economic exploitation. Owing to the vast and varied flora many of these could be profitably utilized for livestock feeding as regular feed only if their nutritive value, palatability and toxic constituents are known. Several such plants might contain deleterious constituents, which could render them unfit for livestock feeding but in many such cases the toxic substances could be eliminated by simple physico-chemical techniques, thereby, making them suitable for consumption by the livestock. Recent investigations of the Indian Grassland and fodder Research Institute have shown that some of

these are quite promising and may have potential use in agriculture and livestock productivity.

The present investigation is confined on the three plants viz. Alysicarpus longifolius Wright and Arn. (Leguminosae), Alysicarpus bupleurifolius DC and Lindenbergia urticaefolia Lehm. (Scrophulariaceae), found in the Bundelkhand region. First two are reported to be nonconventional forage[34] legumes and are medicinally important. The third plant is a local weed and toxic to livestock though described as medicinally useful.

Modern instrumental techniques play a very important role in the chemical investigation of natural plant products. A brief and relevant account of some of the modern techniques used in the present investigation is given below.

Mass spectroscopy is a well established and an essential analytical tool in the structure elucidation of unknown natural products.[35] Rapid developments in the technique of ionisation have resulted in a variety of ways in which ions can be generated from an organic compound.[36] Of all the methods so far used the conventional electron impact ionisation is far the most common. However, certain compounds do not give peaks corresponding to molecular ions in their electron impact mass spectra and thus molecular weight information is not obtainable in such cases. The advent of chemical ionisation mass spectroscopy has partly solved this problem.[37] Similarly the emergence

of field ionisation and field desorption mass spectrosopy has facilitated the analysis of complex mixtures[38] without prior separation and thermally labile and nonvolabile samples[39-40] such as glycosides could be studied without derivatisation.

As in case of hydrocarbons, the electron impact mass spectra shows the presence of peaks at 14 mass intervals due to $C_nH^+_{2n+1}$ ions.[41] The molecular ions are of very low abundance. The presence of oxygen in an aliphatic compound (eg. alcohol) is indicated by the presence of a series of ions corresponding to the formula $C_nH_{2n+1}O^+$. Aliphatic alcohols produce spectra resembling that of hydrocarbons due to the loss of water resulting in the formation of an olefin. Hence, apart from $C_nH^+_{2n+1}$, $C_nH_{2n+1}O^+$ series $C_nH^+_{2n}$ and $C_nH^+_{2n-1}$ series of ions are also seen. However, the chemical ionisation, field ionisation and field desorption mass spectra of these compounds produce peaks due to $(M-H)^+$, M^+ or $(M+H)^+$ and thus help in finding out the molecular weight of these compounds.

The application of nuclear magnetic resonance spectroscopy is also well established. It is today accepted as one of the most powerful physical methods available to the chemist and is considered to be an essential technique for structure elucidation of unknown organic compounds.

Investigations into many branches of chemistry and physics rely to an considerable extent on the analysis of

absorption spectra in the ultraviolet region.[42] In practice, it is for the most part limited to conjugated system. There is however, an advantage to the selectivity of ultraviolet absorption. Characteristic groups may be recognised in molecules of widely varying complexities. It is widely used in the study of natural products. Infrared radiations refer broadly to that part of the electromagnetic spectrum between the visible and the microwave region.[43] The ordinary infrared region extends from 2.5 to 15 μ (4000 to 667 cm^{-1}), the region from 0.5 to 2.5 μ (12,500 to 4000 cm^{-1}) is near infrared and the region from 15 to 200 μ (667 to 50 cm^{-1}) is far infrared. The functional group present in a molecule have characteristic absorption bands and these bands are quite informative in the structure elucidation of these compounds.

Gas chromatography[44] is one of the most powerful separation, identification and determination tool available today. The distribution process effecting a GLC separation is the partition of the solute between a stationary liquid and a mobile gas. A sample mixture on to the GLC column is swept by a carrier gas and after passing through the column the components emerge one after the other. It is widely used for the analysis of enormously complicated mixtures[45] of organic compounds like hydrocarbons, alcohols, esters etc. A recent advancement of GLC is the introduction of high pressure liquid chromatography wherein the separation is

effected by the application of high pressure. HPLC[46] is particularly suited to the analysis of those compounds which are not readily handled by GLC for e.g. thermally labile compounds can be analysed at ambient temperatures by HPLC, highly polar compounds can be chromatographed without prior derivatisation and polymeric samples can also be analysed.

However, such techniques are highly sophisiticated and are of immense value in the analysis of organic compounds.

Chapter-I deals with the chemical investigations of Alysicarpus longifolius. The hexane fraction of the leaves contained a homologous series of alkanes C_{27}-C_{33}, a long chain aliphatic ester and alcohol, sterol and sterol derivative. The plant was found to be rich in flavonoid glycosides. Five glycosides were isolated from ethanol, ethyl acetate and butanol fractions. The butanol fraction also gave free sugars and sugar alcohol.

Detailed investigation of Alysicarpus longifolius seed oil revealed that its oil resembles palm oil. It is high in palmitic acid (49.1%) and displays a high degree of saturation (66.4%). The UV spectrum showed low level of conjugated dienoic acid.

The chemical investigation of Alysicarpus bupleurifolius is dealt within Chapter-II. Systematic analysis of different fractions showed the following classes of compounds. Hexane fraction gave a homologous series of alkanes (C_{27}-C_{35}) and

alcohols (C_{28}-C_{34}). Benzene fraction gave sterol glycoside. Free sugars and sugar alcohols were found in more polar fractions. The plant also contained free essential and non essential amino acids. The presence of similar pattern of hydrocarbons and sugar alcohol-pinitol in the two species of genus Alysicarpus may be of chemo-taxonomic interest.

Chapter-III deals with the chemical investigation of Lindenbergia urticaefolia. As in the previous cases this plant also contained long chain hydrocarbons, free sterol, sterol ester and glycoside, sugar alcohol, flavone and free sugars.

CHAPTER-I

THE CHEMICAL INVESTIGATION OF

ALYSICARPUS LONGIFOLIUS WRIGHT & ARN.

INTRODUCTION

Alysicarpus longifolius Wright and Arn. (Leguminosae) (*Marathi*: Jangli gailia, Motha dampta, *Gujarati*: Dhodasamervo, *local*: Girgaua) belongs to one of the ten known species of genus *Alysicarpus*.

Distribution:

Found throughout the plains of India and Ceylon.

Botanical description and uses:

A glabrous, terete, slightly striate herb; leaves 1-foliate, shortly petioled, subcordate, mucronate, hairy on the veins beneath, stipules large scarious; flowers in long densely spicate racemes, appressed to the finely hairy rachis; bracts large and conspicuous, caducous; calyx pubescent, teeth much larger than the funnel shaped plicate tube; pods shortly stalked, apiculate joints 4-6 glabrous.

Some of the sister species of genus *Alysicarpus* like *A.pubescens* Law. *A.rugosus* DC. and *A. longifolius* Wright & Arn. are good pasture legumes.[47] The roots of *A.longifolius* have been reported to be used as substitute for liquorice.[48-49]

PREVIOUS WORK:

The literature survey has revealed that the plants of this genus are almost phytochemically unexplored. *A. vaginalis*[50] has been reported to contain carotene and vitamin C. Proximate analysis and the seasonal variations of crude protein, ash, dry matter in the leaves of *A.rugosus* have been described by Indian workers.[51]

Alysicarpus longifolius

PRESENT WORK:

In view of its usefulness as a nutritious fodder plant of Bundelkhand region and its medicinal importance it was considered of interest to undertake a detailed chemical investigation of A.longifolius. Air - dried powdered leaves of A.longifolius were extracted with ethanol (95%). The combined extract was separated into hexane, benzene, ethyl acetate, acetone and n-butanol soluble fractions. Each fraction was investigated in detail. The column chromatography of these fractions over silica gel followed by preparative TLC afforded the following substances as shown in Table-1.

Table-1

Sl. No.	Name of Constituents	Molecular formula	m.p.
1.	Substance A		65-67°
	a) Heptacosane	$C_{27}H_{56}$	
	b) Octacosane	$C_{28}H_{58}$	
	c) Nonacosane	$C_{29}H_{60}$	
	d) Triacontane	$C_{30}H_{62}$	
	e) Hentriacontane	$C_{31}H_{64}$	
	f) Dotriacontane	$C_{32}H_{66}$	
	g) Tritriacontane	$C_{33}H_{68}$	
2.	Substance B Carnaubyl cerotate	$C_{50}H_{100}O_2$	72-75°
3.	Substance C Myricyl alcohol	$C_{30}H_{62}O$	85-86°

Sl. No.	Name of Constituents	Molecular formula	m.p.
4.	Substance D β-sitosterol	$C_{29}H_{50}O$	136-37°
5.	Substance E β-sitosterol acetate	$C_{31}H_{52}O_2$	128-29°
6.	Substance F Phegopolin	$C_{22}H_{22}O_{10}$	203-4°
7.	Substance G Rutin	$C_{27}H_{30}O_{16}$	189-90°
8.	Substance H Quercetin 7-O-rhamnoside	$C_{21}H_{20}O_{11}$	222-25°
9.	Substance I Chrysoeriol 7-O-xyloside	$C_{21}H_{20}O_{10}$	-
10.	Substance J Pinitol	$C_7H_{14}O_6$	186-87°
11.	Substance K Kaempferol 3-O-xyloside-7-O-rhamnoside	$C_{26}H_{28}O_{14}$	237° (dec)
12.	Free sugars		
	a) Glucose		
	b) Xylose		

RESULTS AND DISCUSSION

Substance A: m.p. 65-67°. Obtained as waxy flakes, which crystallised from benzene as colourless microcrystalline solid. GLC analysis showed it to be a mixture of mainly seven components (Fig.1). The IR spectrum of substance A showed bands only for C-H stretching and C-H bending vibrations suggesting it to be hydrocarbons[52] (Fig.2). The electron impact mass spectrum (EIMS) showed a series of peaks at odd mass corresponding to $C_nH^+_{2n+1}$ ions and prominent even mass ion peaks at m/z 380, 390, 408, 422, 436, 450 and 464 (Fig.3). This further showed that substance A is a mixture of seven straight chain hydrocarbons. As field ionisation mass spectrum (FIMS) give only molecular ions with little or no fragmentation the FI spectrum of substance A (Fig.4) was obtained to confirm the molecular weights and thus the identity of the components. Peaks at m/z 380, 394, 408, 422, 436, 450 and 464 were observed, thus confirming the molecular weights of the components. Thus substance A was identified as a mixture of the following hydrocarbons[53-57] i.e. heptacosane(1), octacosane(2), nonacosane (3), triacontane (4), hentriacontane (5), dotriacontane (6) and tritriacontane (7). The approximate percentage composition of the mixture as found from GLC and FIMS data is presented in Table-2.

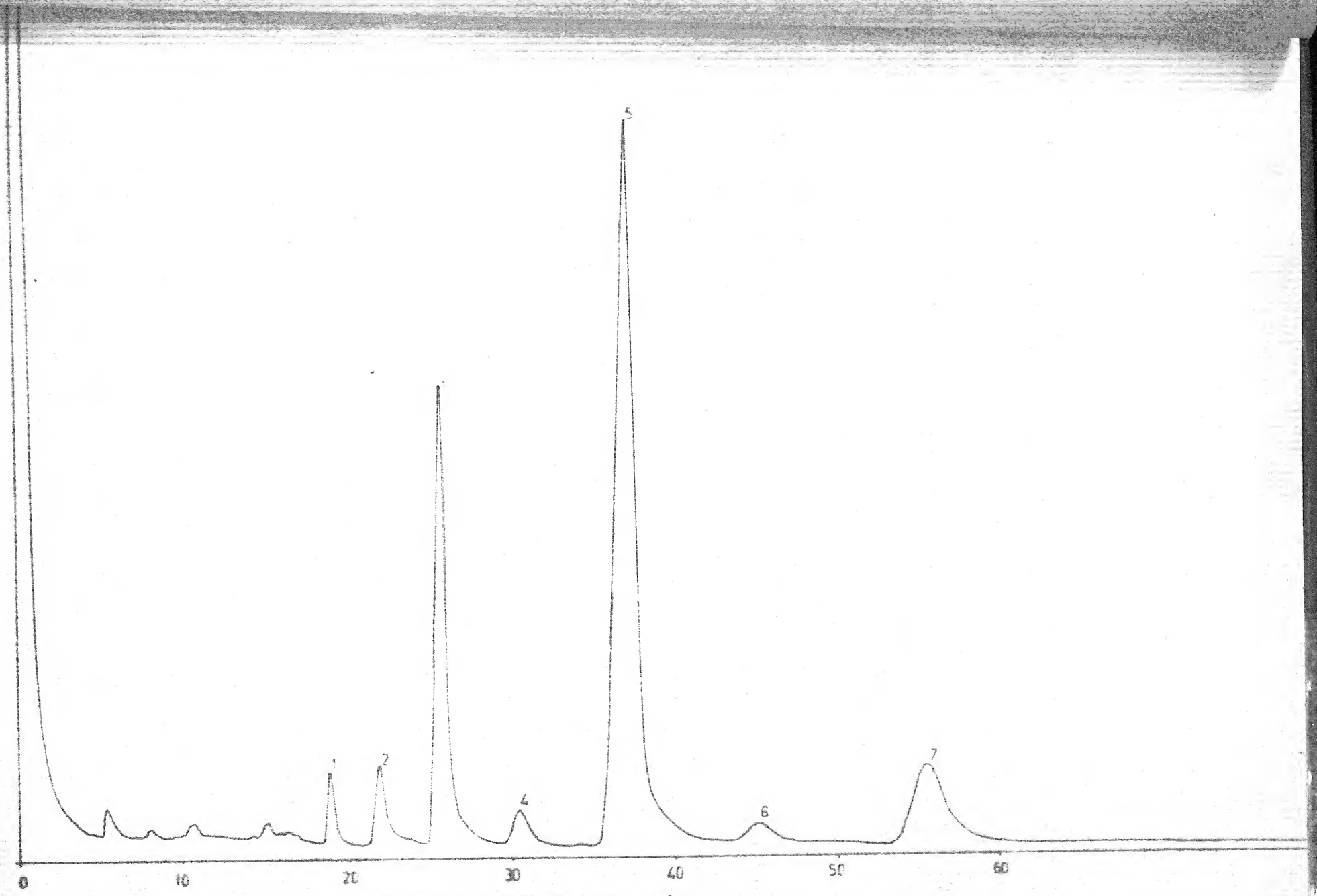


FIG-1 GLC OF SUBSTANCE-A

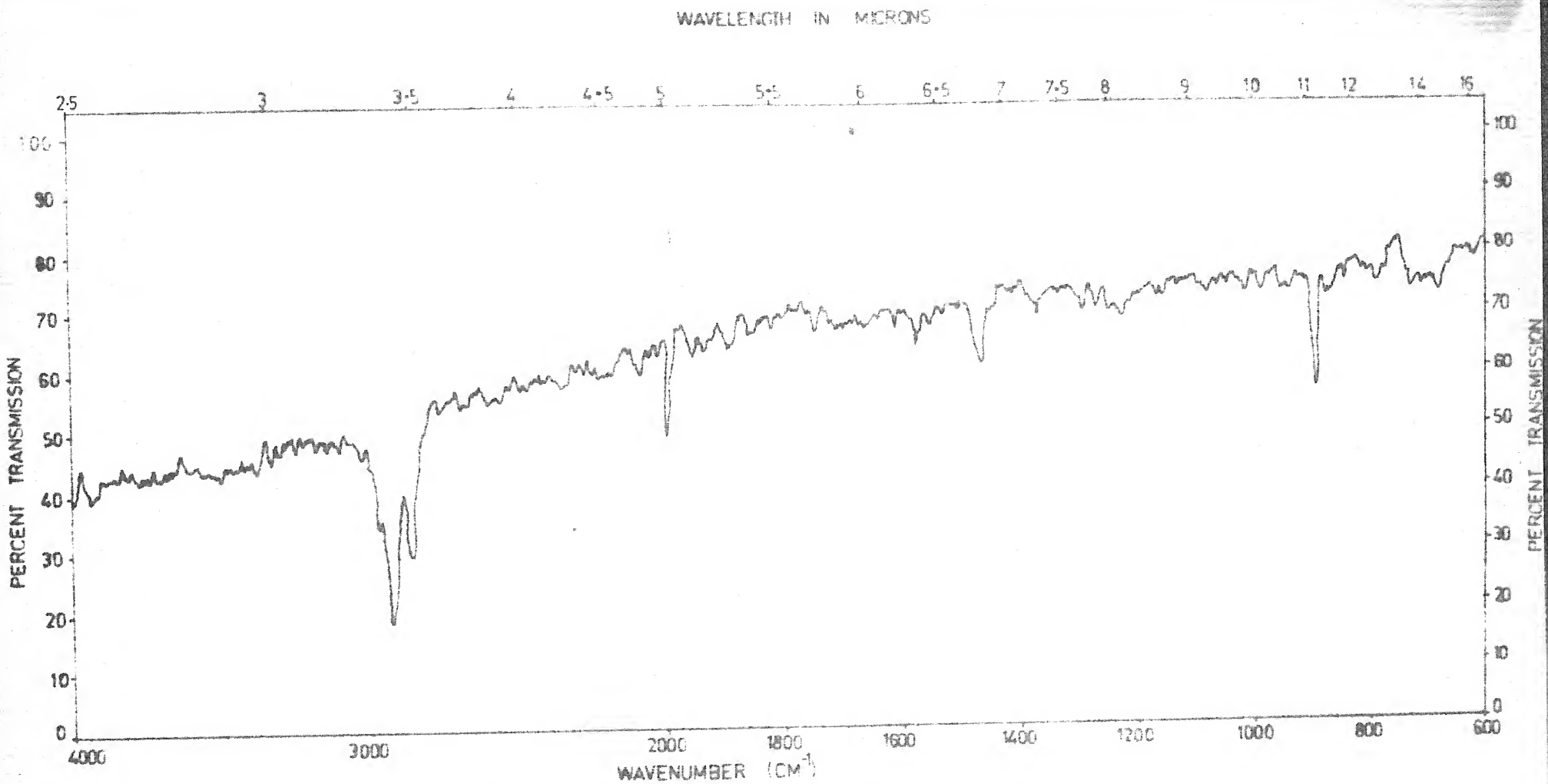


FIG-2 IR SPECTRUM OF SUBSTANCE-A.

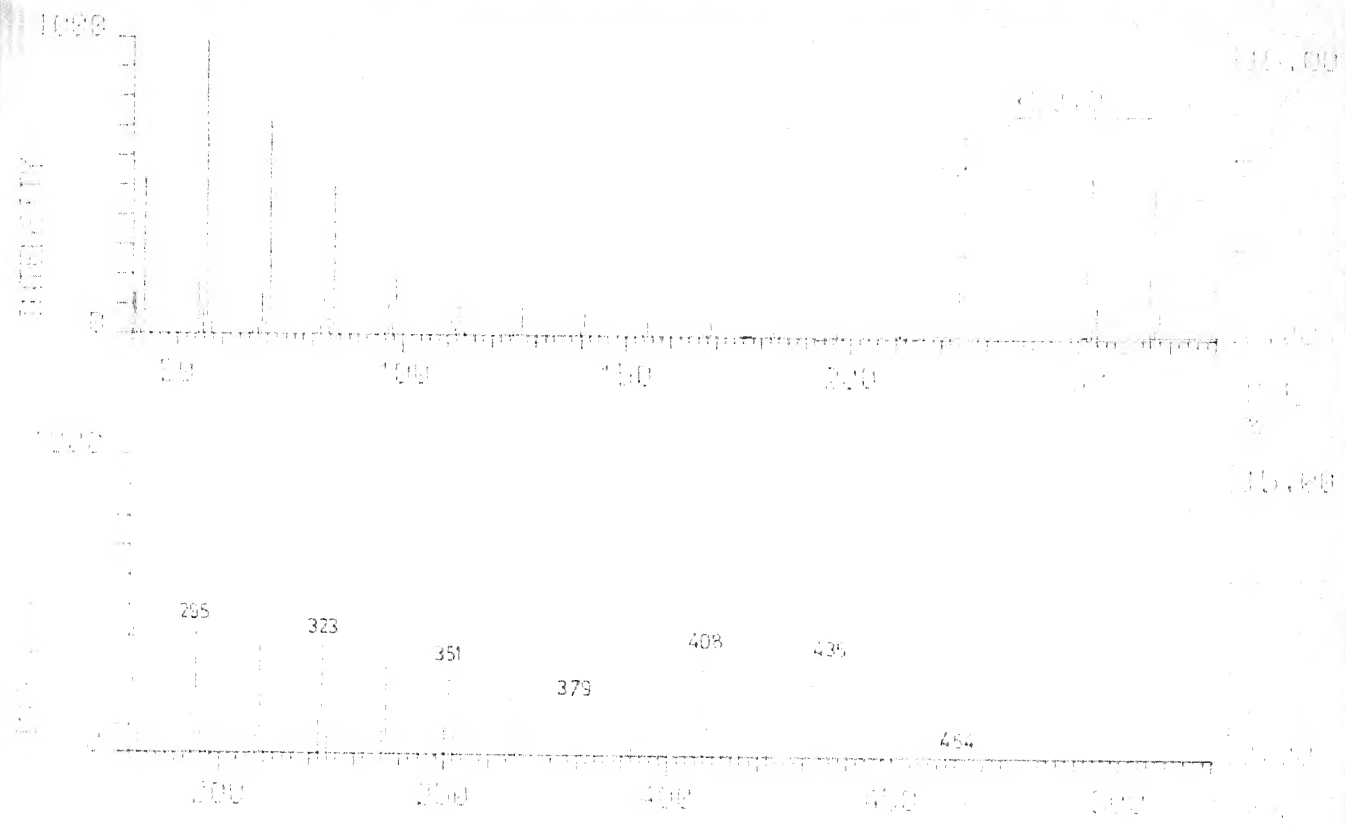


FIG-3 EI MASS SPECTRUM OF SUBSTANCE-A.

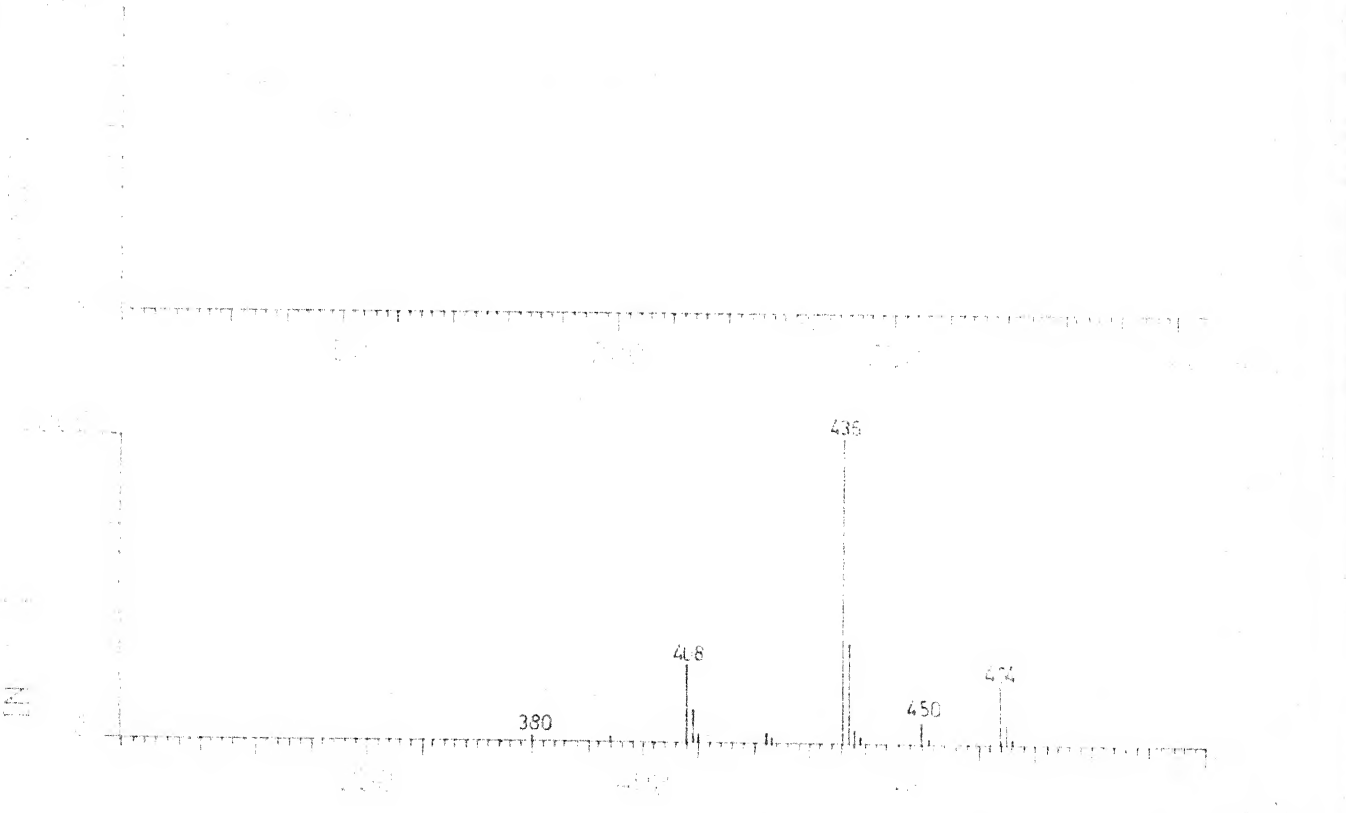


FIG-4 FI MASS SPECTRUM OF SUBSTANCE-A.

Table-2

Peak No.	GLC DATA %	FIMS DATA %	Molecular Wt.
1	2.1	1.3	380
2	2.8	1.8	394
3	25.6	16.5	408
4.	2.1	2.3	422
5	55.8	62.6	436
6	1.9	4.5	450
7	9.5	10.9	464

Substance B: m.p. 72-75°. Obtained as a white solid from acetone. It was assigned the molecular formula $C_{50}H_{100}O_2$ on the basis of its elemental analysis. The IR spectrum (Fig.5) was suggestive of its aliphatic nature and showed an intense band at 1730 cm^{-1} indicating the presence of a carbonyl group. The NMR spectrum supported its straight chain nature. In its NMR spectrum (Fig.6) the two terminal methyl protons appeared as a deformed triplet at δ 0.85 and δ0.65. A singlet integrating for 90 protons at δ1.20 was due to the presence of $-(CH_2)_{45}-$ chain. The two triplets characteristic of methylene protons attached to oxygen atom ($-OCH_2-$) and to carbonyl group ($-CH_2-CO$) appeared at δ3.80 and δ2.26 respectively. The mass spectrum (Fig.7) showed a molecular ion peak at m/z 732 and a series of

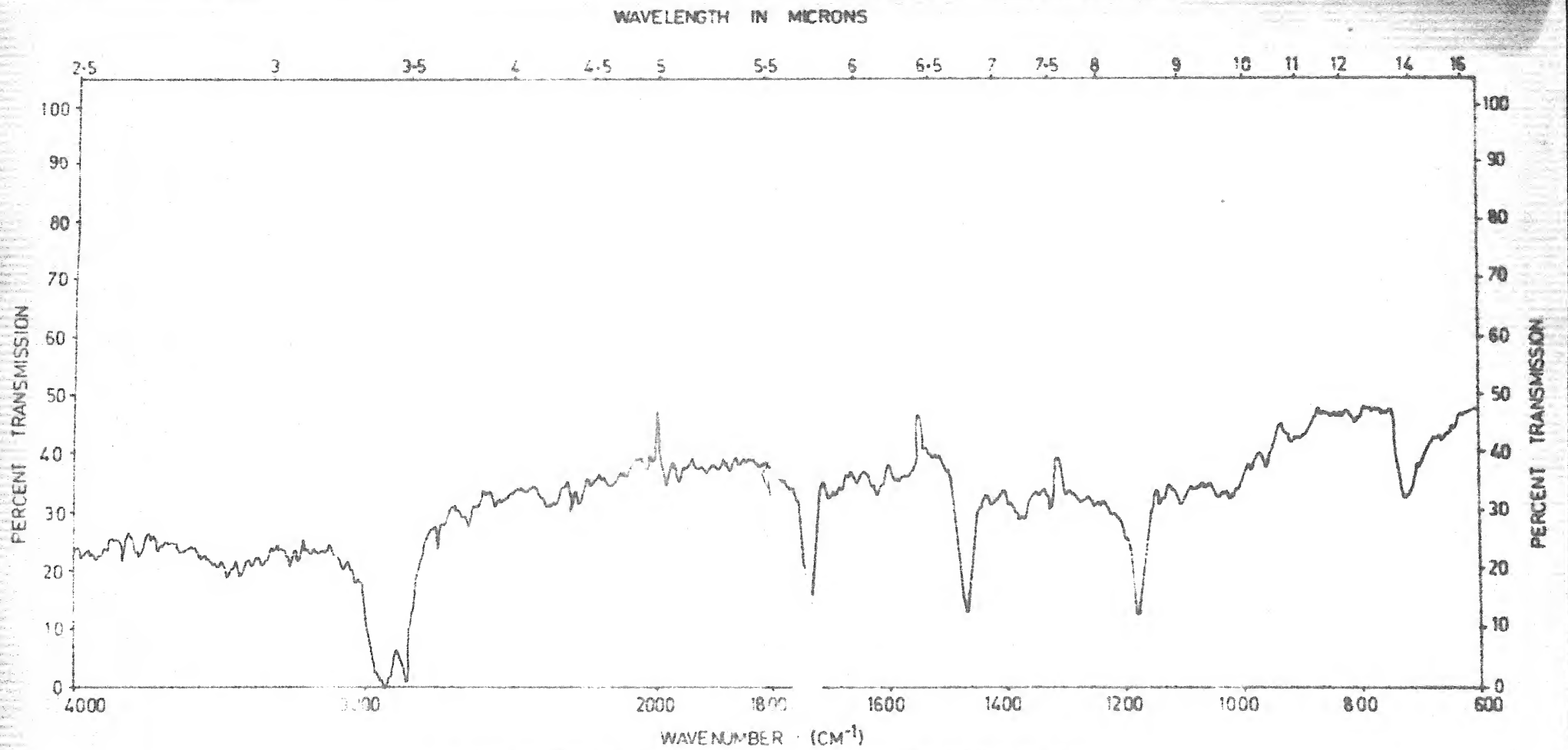


FIG-5 IR SPECTRUM OF SUBSTANCE-B.

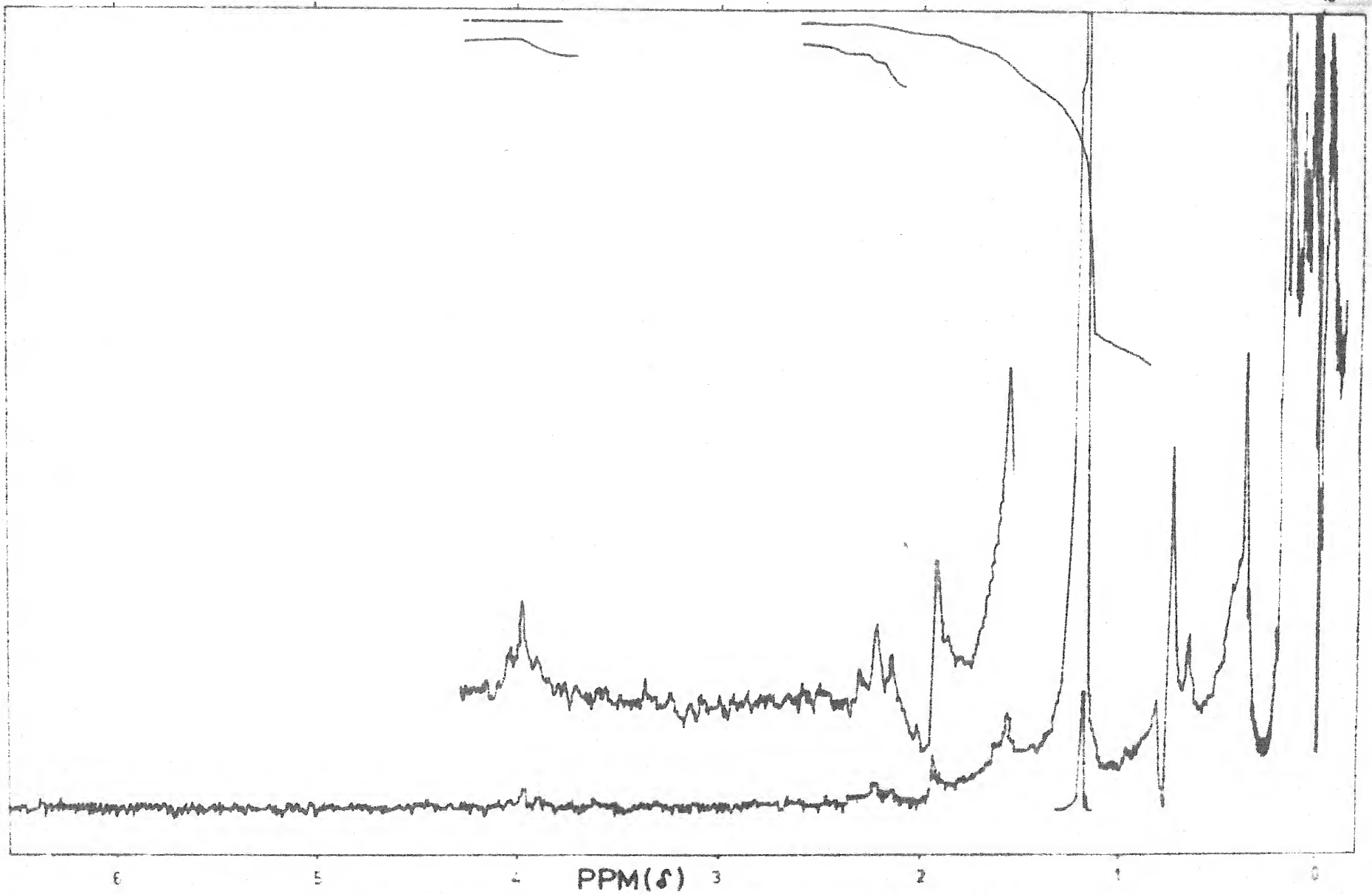


FIG-6 NMR SPECTRUM OF SUBSTANCE-B.

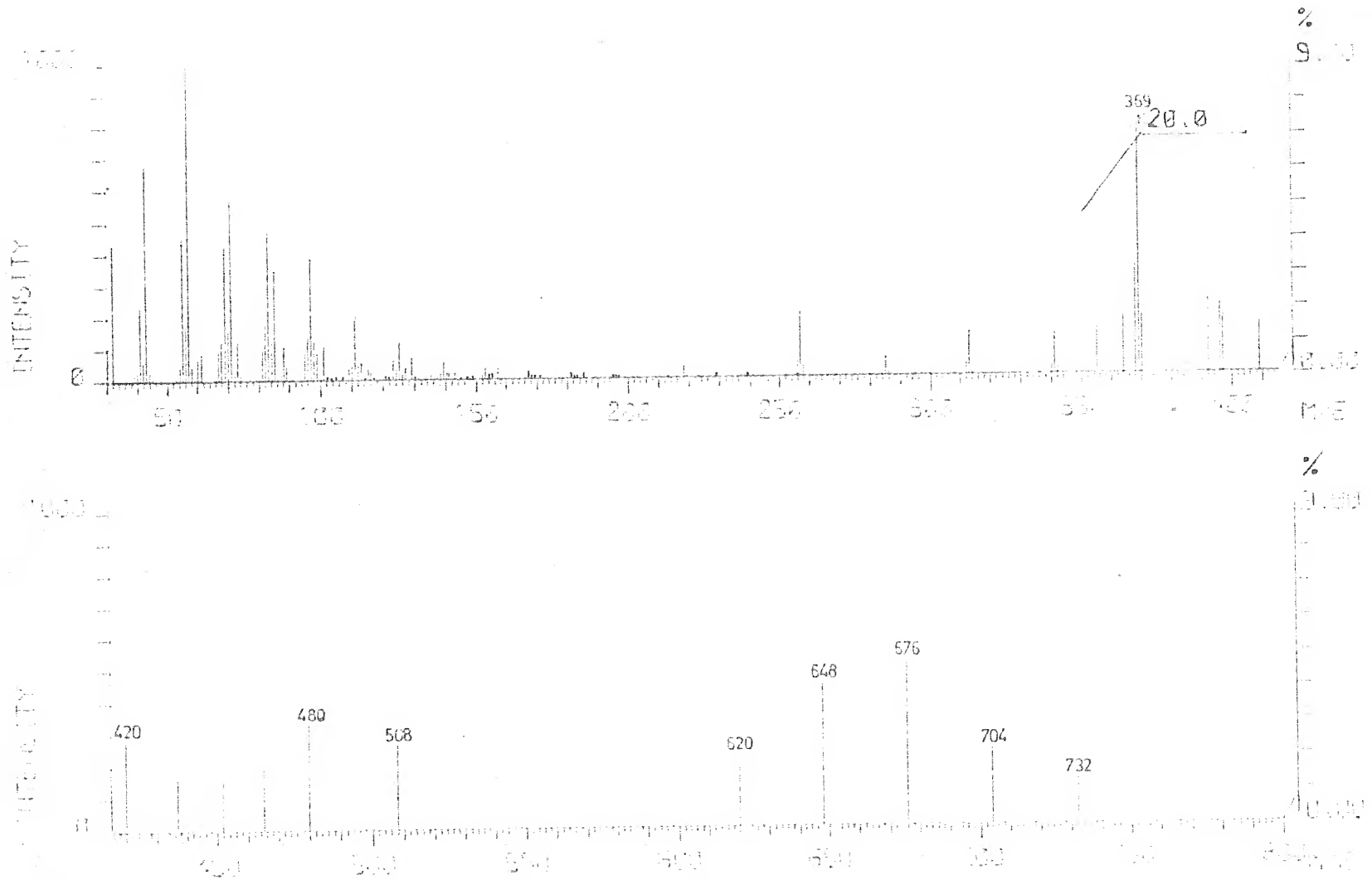


FIG-7 EI MASS SPECTRUM OF SUBSTANCE-B.

peaks corresponding to $C_nH^+_{2n+1}$ and $C_nH^+_{2n-1}$ ions suggesting it to be a long chain aliphatic compound.

The substance B was identified as an ester by its alkaline hydrolysis which yielded two products (9) and (10) characterised as carnaubyl alcohol ($Me(CH_2)_{21}CH_2OH$), m.p. 68°(MeOH) and cerotic acid ($Me(CH_2)_{25}CO_2H$), m.p. 75-76° (ethyl acetate) respectively by comparison with their authentic samples. The substance B was thus identified as carnaubyl cerotate [58] (8) and its physico-chemical data corresponded to the reported values in the literature.

Substance C: m.p. 85-86°. Crystallised from benzene. It was analysed for $C_{30}H_{62}O$. The appearance of bands at 3448 and 1053 cm^{-1} in its IR spectrum indicated the presence of hydroxy function. The long chain aliphatic nature in substance C was evident from its spectroscopic studies. The fragmentation pattern obtained by mass spectral study showed a series of odd mass ions corresponding to $C_nH^+_{2n-1}$ and $C_nH_{2n+1}O^+$ (Fig.8). The prominent even mass ion peak at m/z 420 might be due to the olefin produced by the dehydration of alcohol. The chemical ionisation mass spectrum (CIMS) (Fig.9) of substance C showed peaks at m/z 421 and 437 which corresponded to $(M+H-H_2O)^+$ and $(M-H)^+$ respectively. Hence the molecular weight of substance C appears to be 438. Finally the molecular Wt. was confirmed by field desorption mass spectrum (FDMS). The NMR spectrum (Fig.10) showed a triplet at δ 0.89 for one terminal methyl group and a broad singlet at δ 1.20 integrating for 56 protons.

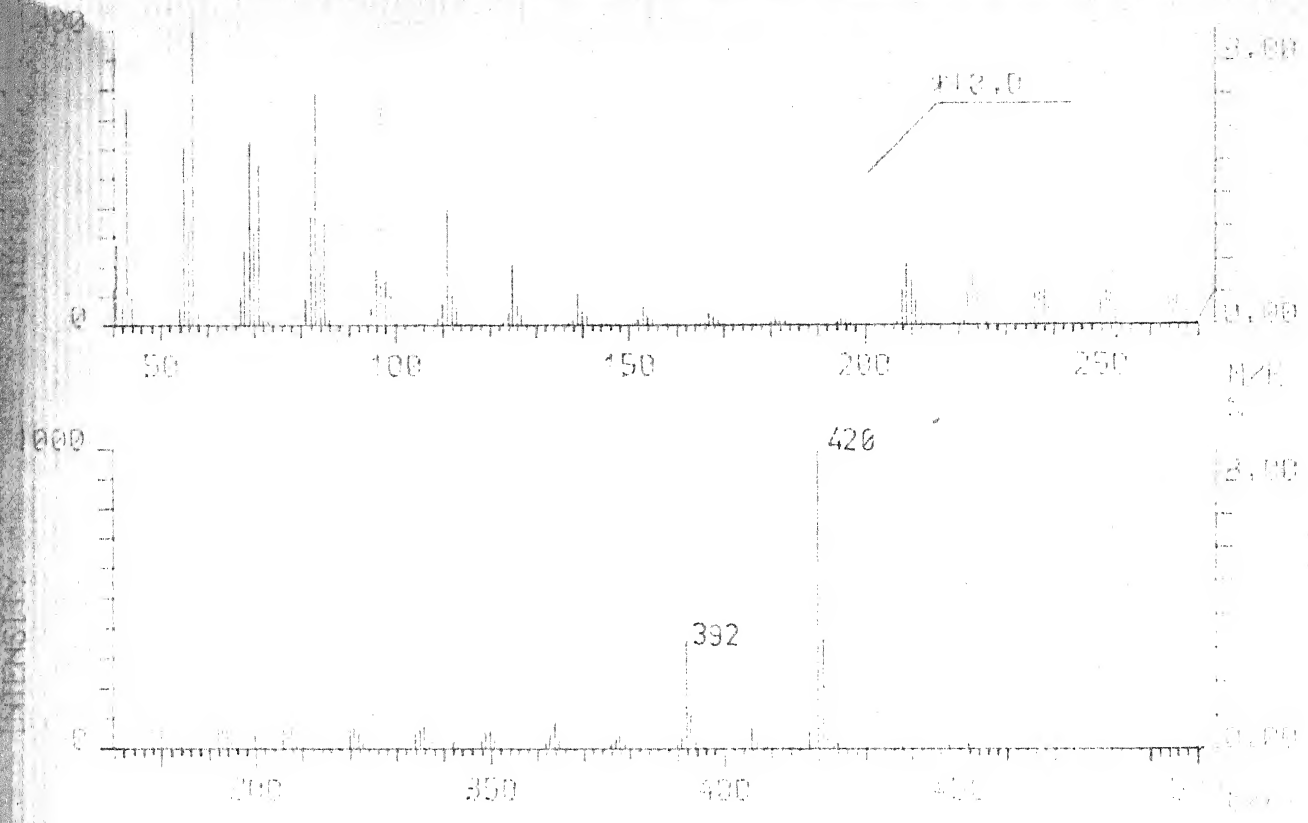


FIG.-8. EI MASS SPECTRUM OF SUBSTANCE-C.

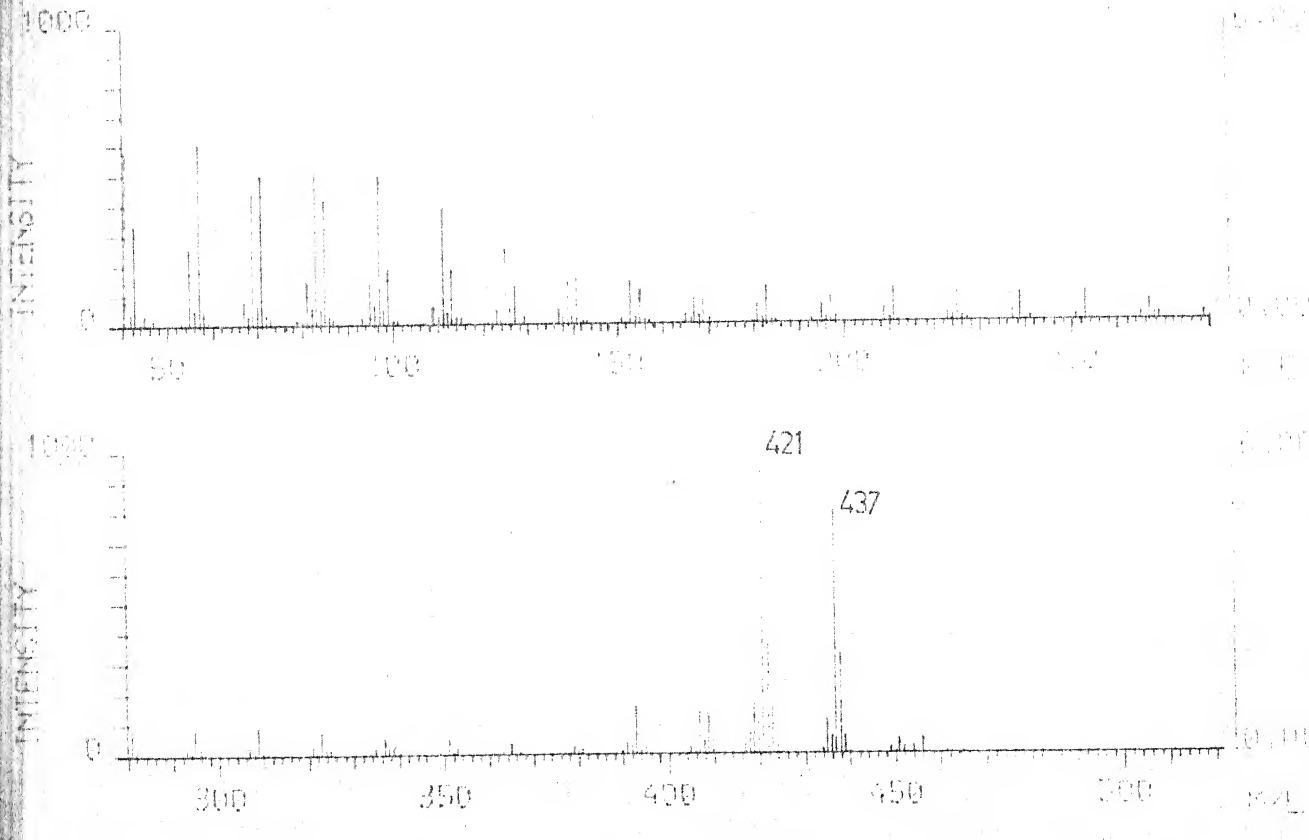


FIG-9. CI MASS SPECTRUM OF SUBSTANCE-C.

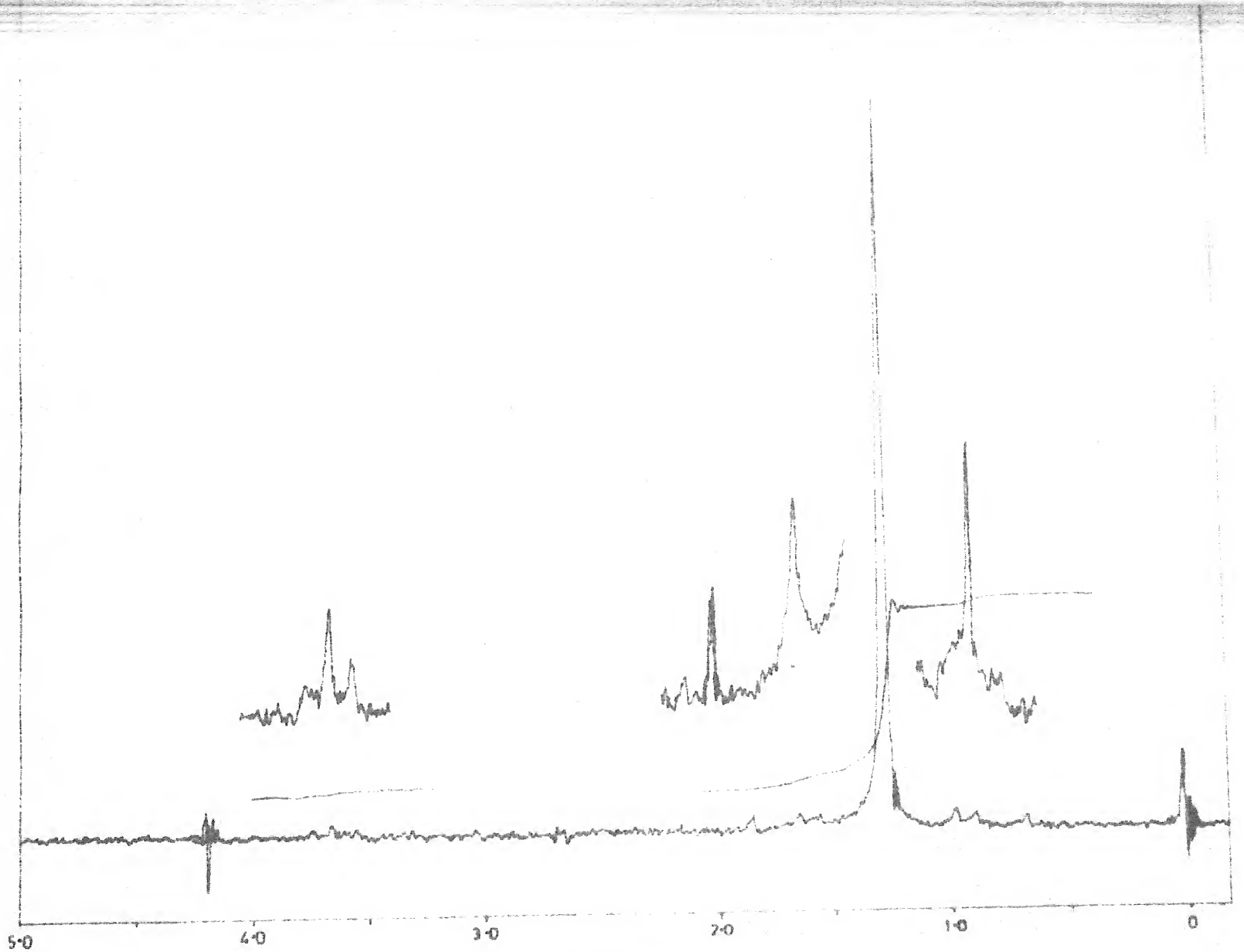


FIG.-10 NMR SPECTRUM OF SUBSTANCE-C.

due to $(-CH_2)_{28}$-chain. The two methylene protons attached to the oxygen atom ($-\underline{CH}_2-OH$) appeared as triplet at δ 3.65. The substance C formed an acetate. The IR spectrum of acetate showed a band at 1730 cm^{-1} for the acetoxy function. The appearance of a sharp singlet at δ 2.05 in the NMR spectrum (Fig.11) further confirmed the presence of acetoxy group. The mass spectrum of the acetate exhibited molecular ion peak at m/z 480 (M^+). All the physico-chemical data of substance C and its acetate were found to be in conformity with the reported literature values of myricyl alcohol and its acetate respectively. The substance C was thus identified as myricyl alcohol[59-60] (11) and confirmed by direct comparison with an authentic sample.

Substance D: m.p. 136-137°. Crystallised from chloroform-methanol, $(\alpha)_D^{25^\circ}$ - 40° (c, 1, $CHCl_3$). Elemental analysis corresponded to molecular formula $C_{29}H_{50}O$. It gave positive Liebermann - Burchard test [61] for steroids and positive tetranitromethane test [62] for unsaturation which showed that the compound under investigation is an unsaturated sterol. The IR spectrum of compound E displayed the bands at 3400 and 1630 cm^{-1} indicating the presence of a hydroxyl group and a double bond respectively. Its NMR spectrum (Fig.12) showed the presence of six methyl groups as overlapping singlets in the region δ 0.66-1.24. The broad multiplets at δ 5.25, 3.40 and 2.10 were due to the $-C=\underline{CH}-CH_2-$, $C\underline{H}OH$ and $CHO\underline{H}$ protons. The mass spectrum of substance D is shown in Fig.13. Apart from the molecular

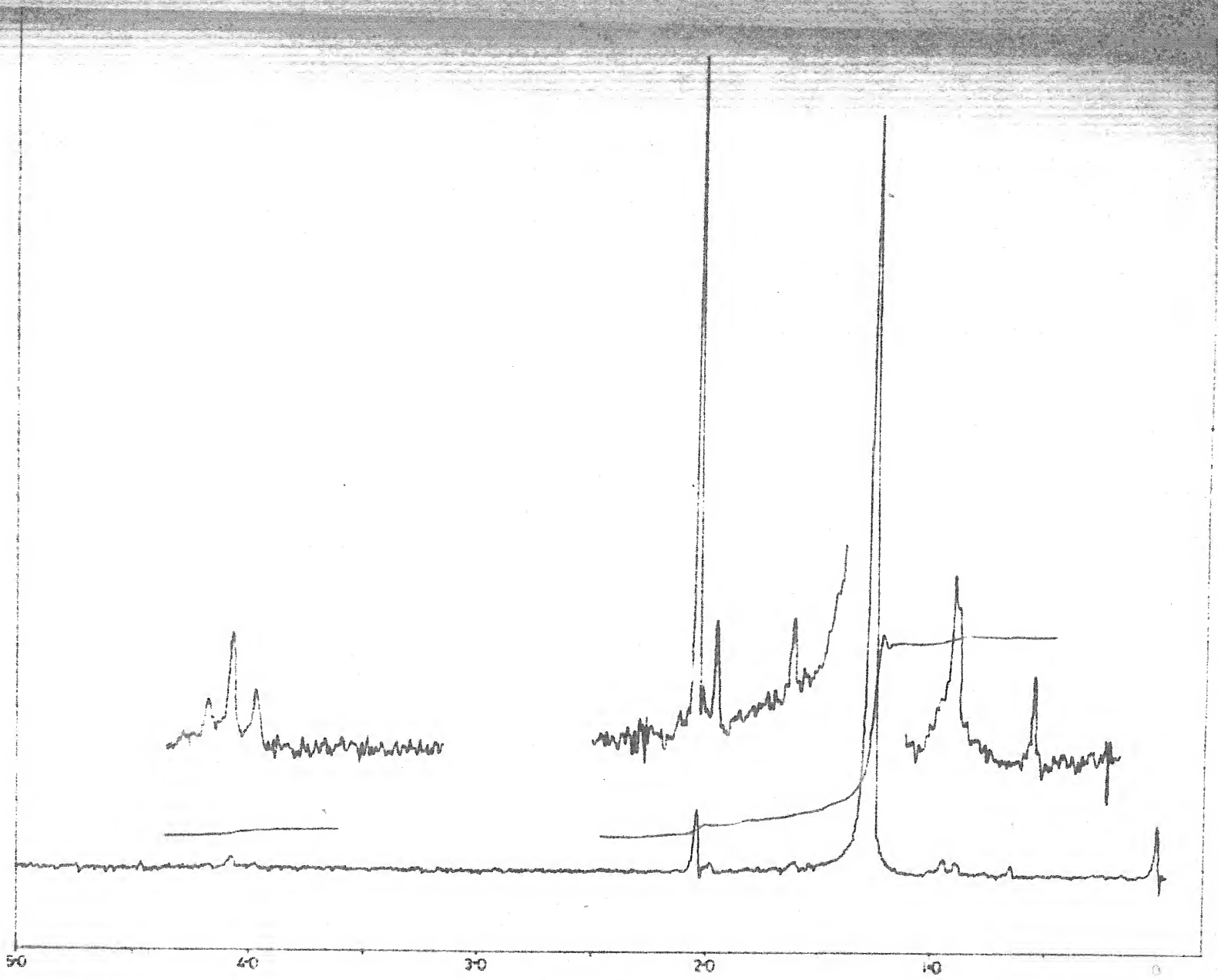


FIG.-11 NMR SPECTRUM - ACETATE OF SUBSTANCE-C.

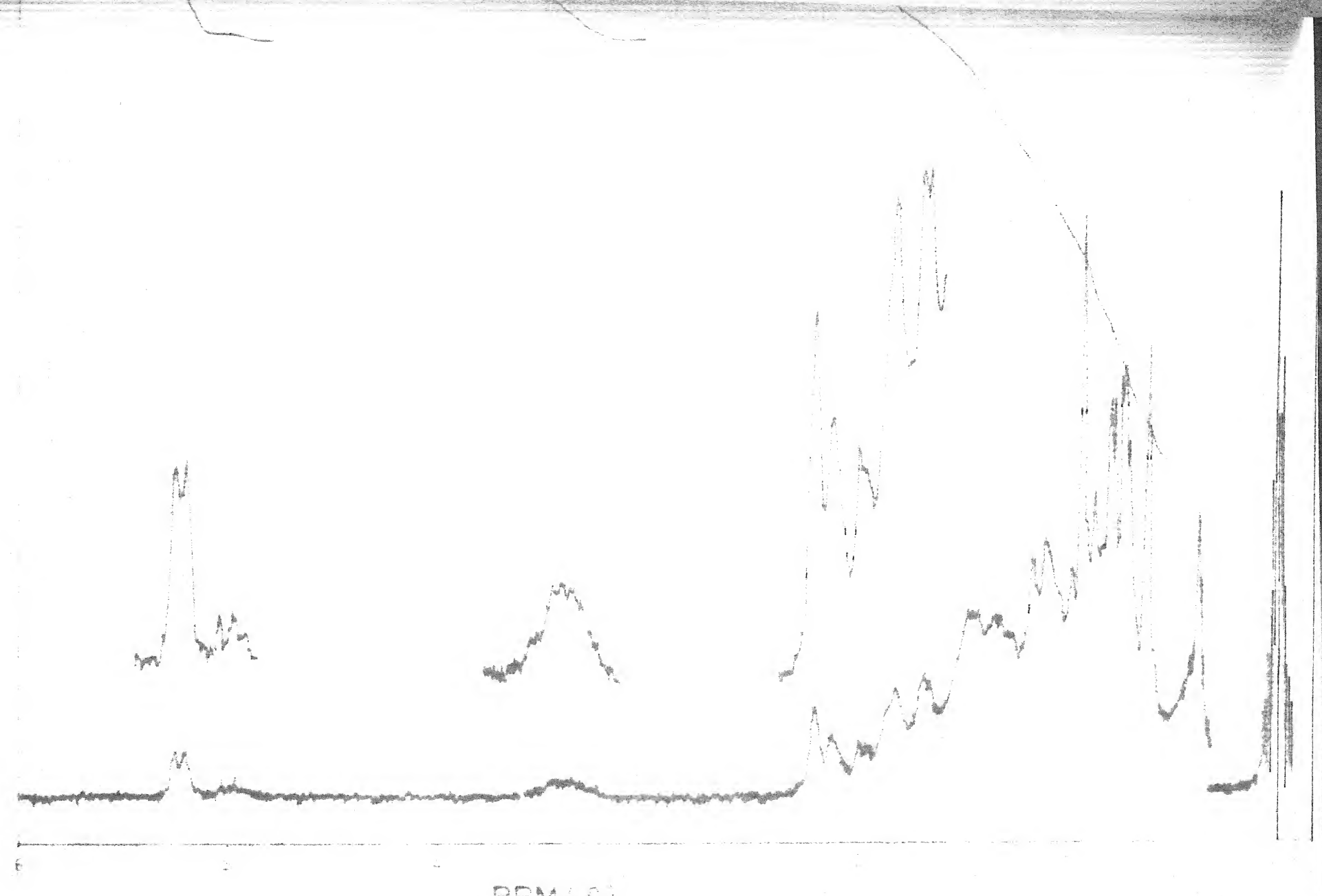


FIG-12 NMR SPECTRUM OF SUBSTANCE D

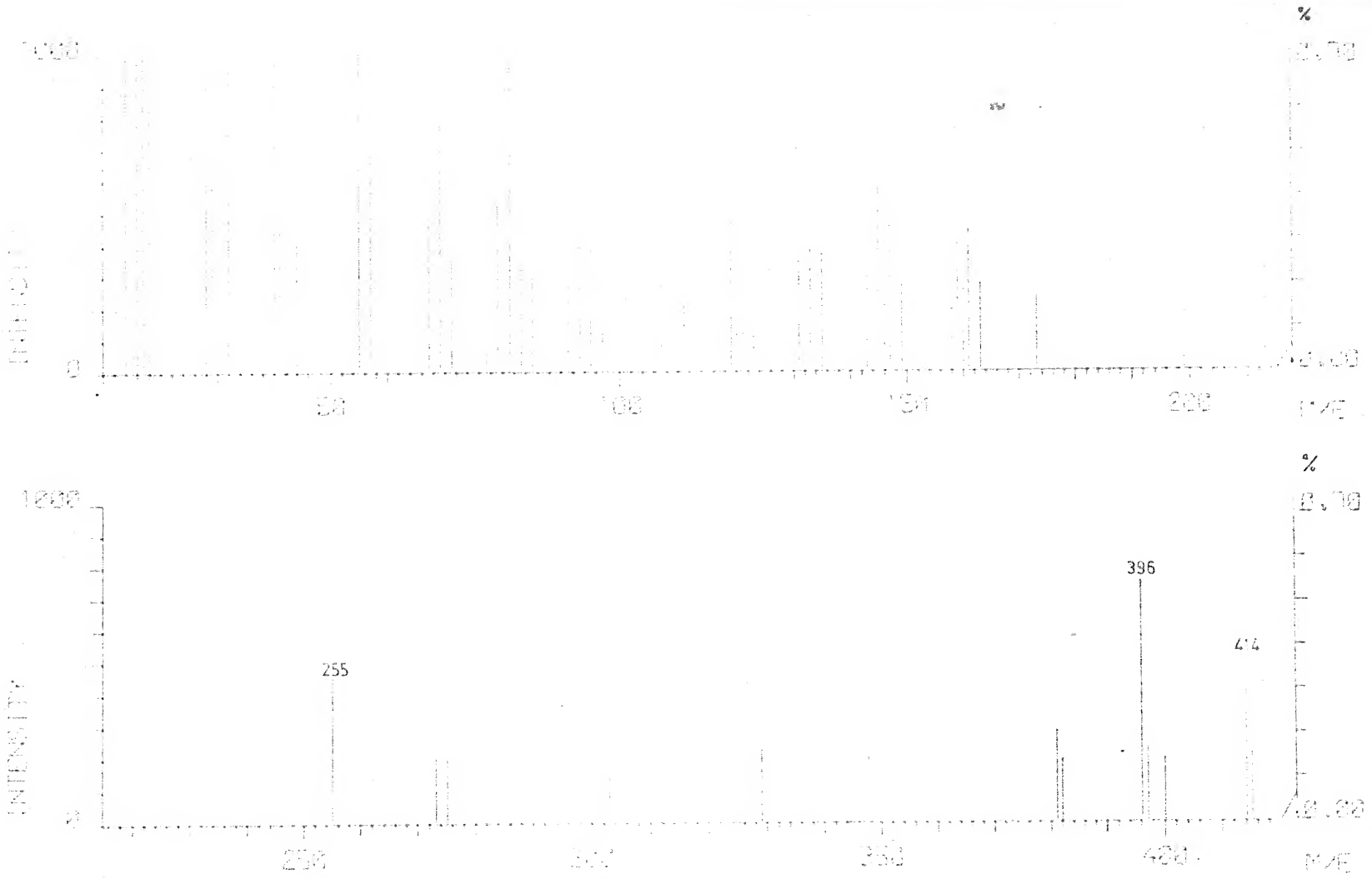


FIG-13 EI MASS SPECTRUM OF SUBSTANCE - D.

ion at m/z 414 prominent peaks were seen at m/z 396 and 255. The substance D formed an acetate. The IR spectrum of acetate showed bands at 1730 and 1260 cm^{-1} for the acetoxy function. The appearance of a sharp singlet δ 1.92 and a multiplet at δ 4.50 in the NMR spectrum confirmed the presence of an acetoxy group and acetoxy methine protons respectively. All the physico-chemical data of the substance D and its acetate corresponded to the reported value of β-sitosterol[60,63] (12). The substance D was further confirmed as β-sitosterol by direct comparison with authentic sample.

Substance E: m.p. 128-129°, $(\alpha)_D^{29°}$ - 34.5° (c, 1, $CHCl_3$), analysed for $C_{31}H_{52}O_2$ and confirmed by the appearance of the molecular ion peak at m/z 456 in its mass spectrum. It gave positive Liebermann-Burchard test indicating it to be a sterol derivative. The IR spectrum displayed an intense bands at 1730 and 1260 cm^{-1} indicating the presence of an acetoxy group. The NMR spectrum appeared to be identical with that of β-sitosterol acetate. The m.m.p, co-TLC, super-imposable IR spectrum of compound E with an authentic sample led to its identification as β-sitosterol acetate[60,63] (13).

Substance F: Crystallised from ethanol as yellow granules, m.p. 203-204°. It developed magenta colour with Mg/HCl (Shinoda test)[64] and intense yellow spot with ammonia suggesting it to be a flavonoid. A positive Fiegel test[65]

indicated its glycosidic nature. Its elemental analysis led to molecular formula $C_{22}H_{22}O_{10}$. The IR spectrum (Fig.14) of compound F showed a broad hydroxyl band at 3400 cm^{-1} and a strong band at 1640 cm^{-1} due to carbonyl group in a pyrone ring. Bands at 1595 and 1555 cm^{-1} indicated the presence of aromatic nucleus and the bands at 1248 and 1060 cm^{-1} showed the presence of ether linkage (Ar-O-C).

The UV spectrum (Fig.15) of compound F showed absorption maxima at 272 and 334 nm. A bathochromic shift of 43nm in band Ia (in $AlCl_3$) relative to band I (in MeOH) indicated a free hydroxyl group at C-5 position.[66] There was no shift in both the bands with addition of sodium methoxide and sodium acetate indicating the absence of hydroxyl groups at C-4 and C-7 position. Since no shift was observed with boric acid buffer solution the possibility of ortho - dihydroxyl function is ruled out. The FD mass spectrum of substance F is shown in Fig.16. The peaks at m/z 447 and 469 corresponded to the $(M+H)^+$ and $(M+Na)^+$ions. Loss of the sugar moiety from $(M+Na)^+$ and $(M+H)^+$ could give rise to peaks at m/z 307 and 285. Hence it appears that the sugar unit has a molecular weight of 180. In the EI mass spectrum of substance F the major peaks were at 284 $(M-162)^{\cdot+}$, 121, followed by retro Diels-Alder (RDA) cleavage ion at m/z 166 and peaks at 167, 138 and 118. The major fragment at m/z 121 suggested that sugar is present in the B ring (Scheme-1).

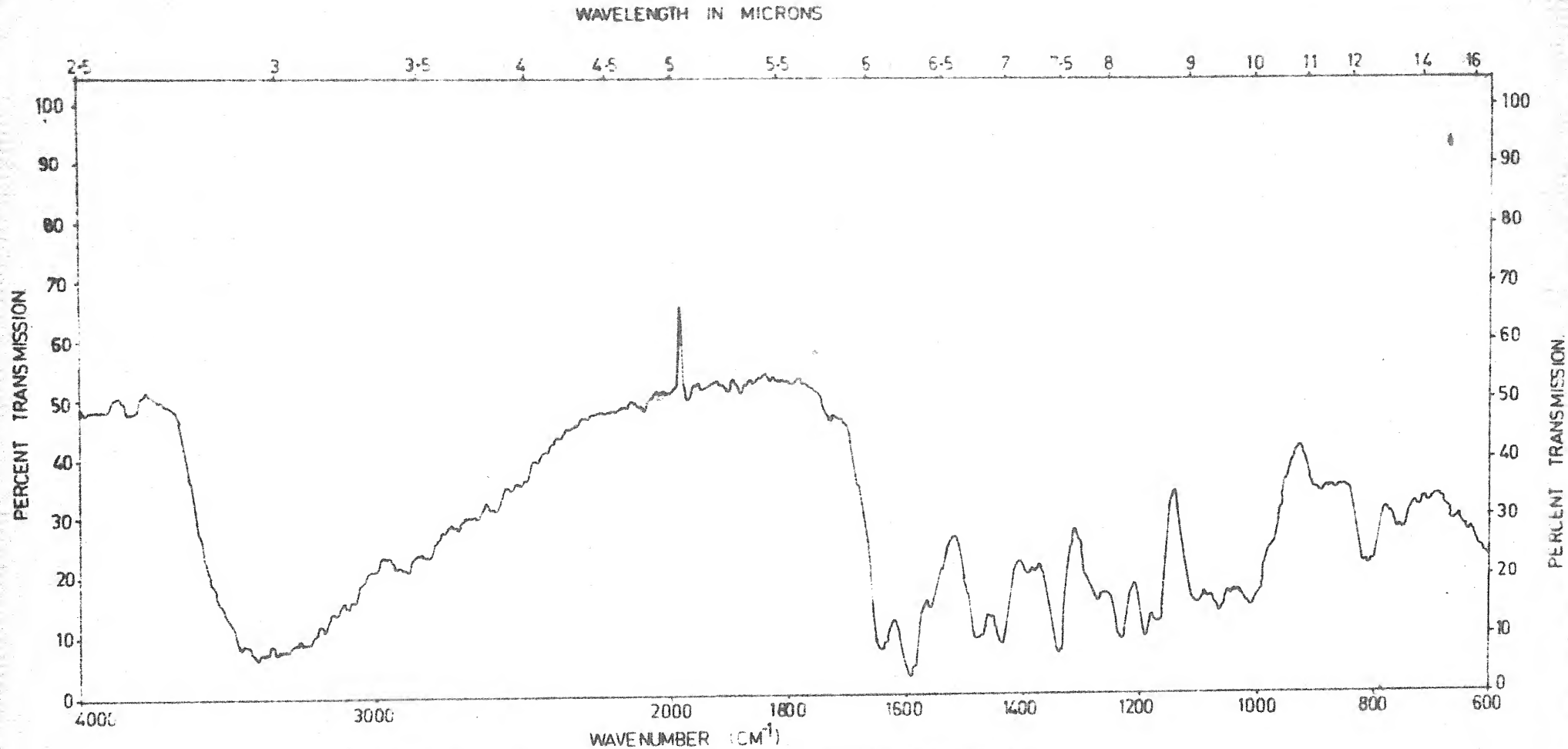


FIG-14 IR SPECTRUM OF SUBSTANCE-F.

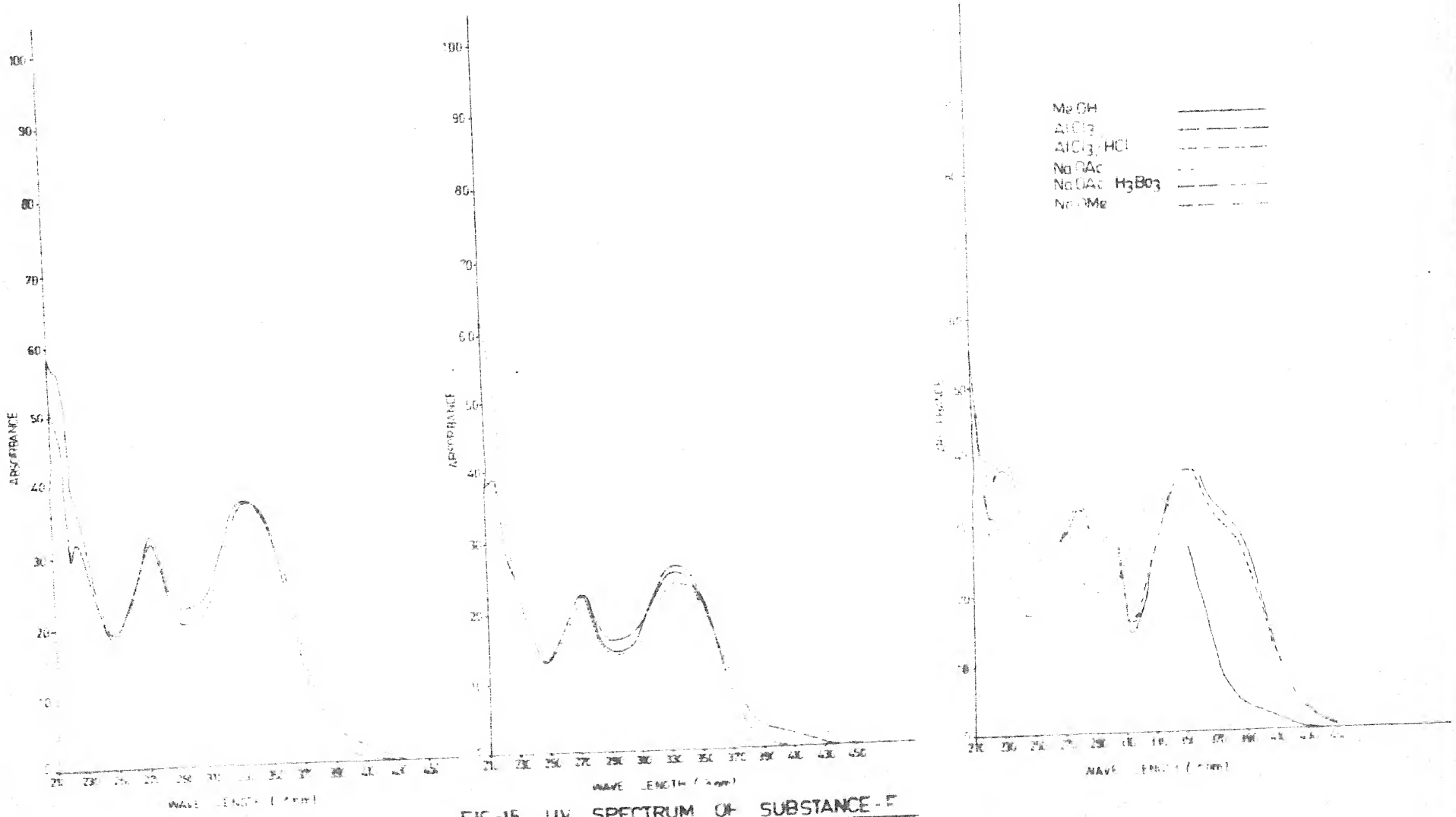


FIG-15 UV SPECTRUM OF SUBSTANCE-F

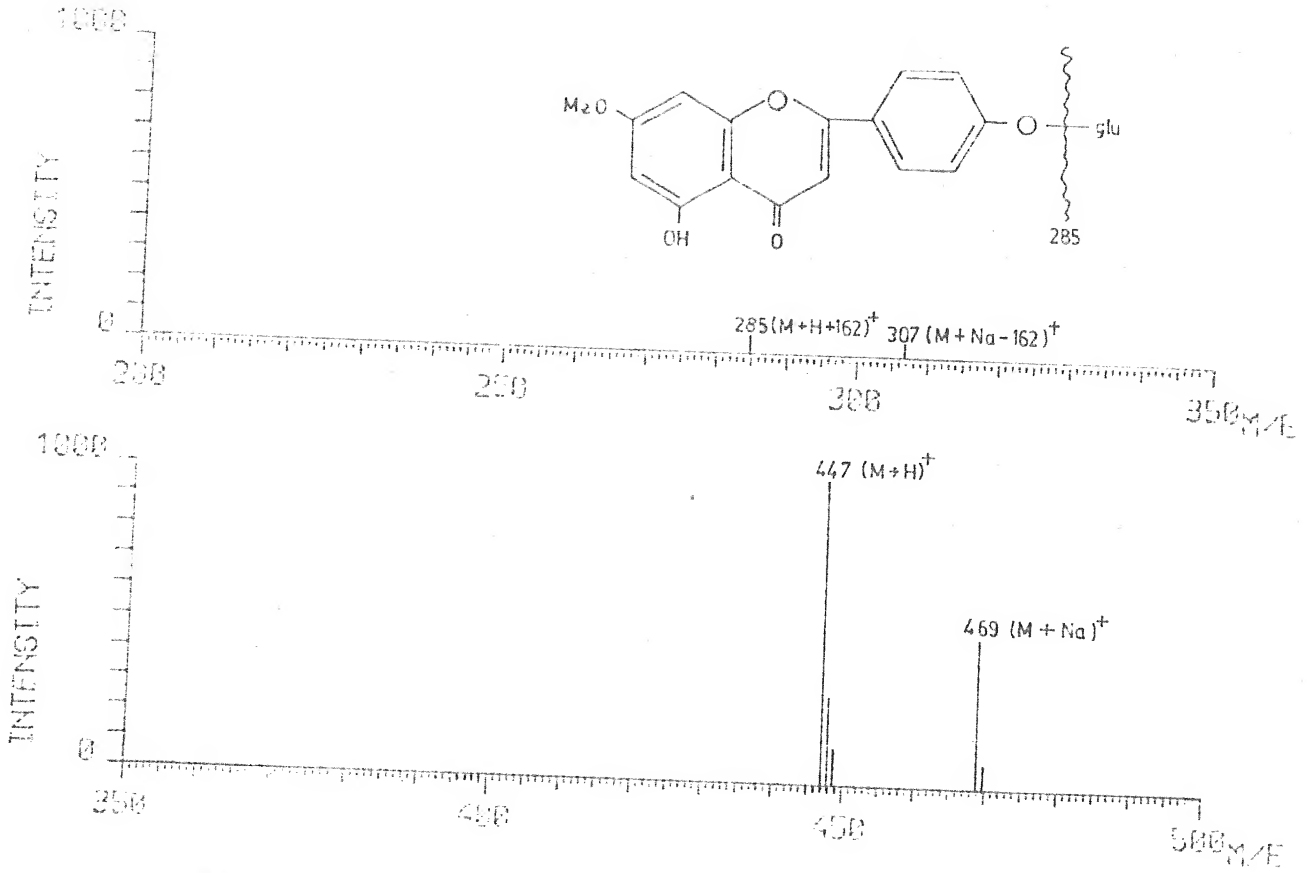


FIG-16 FD MASS SPECTRUM OF SUBSTANCE-F.

It formed a penta acetate the IR spectrum (Fig.17) of which exhibited an intense band at 1760 cm^{-1} due to acetyl carbonyl and was devoid of hydroxyl absorption. In the NMR spectrum (Fig.18) of the acetate the five acetyl signals appeared at δ 2.24, 1.98, 1.92 and 1.69 and $OC\underline{H}_3$ appeared as singlet at δ 3.86. The six sugar protons (H-2",6") resonated in the region δ 4.9 - 5.35 as multiplet. The anomeric proton appeared as doublet at δ 5.75 (J= 2.0 Hz). The aromatic protons appeared at δ 6.34, 6.50 (2H, AB system, J= 2.5 Hz , ring A protons H-6 and H-8). and 7.13 and 7.75 (4H, A_2B_2 system, J= 10.0 Hz ring B protons H-2', 6' and H-3', 5'). The NMR spectrum clearly showed the 5,7 di-substitution in ring A and 4' substitution in ring B.

On acid hydrolysis of compound F, an aglycone was obtained which crystallised from methanol, as yellow granules m.p. 280°. The mass spectrum showed a molecular ion peak at m/z 284. It responded to Shinoda test. Its identity as genkwanin[67] (15) was confirmed by co-TLC and m.m.p. with an authentic sample.

The aqueous portion after neutralisation was found to contain glucose by paper chromatography alongwith an authentic sample in butanol-acetic acid-water (4:1:5,v/v,upper) as developing solvent and aniline hydrogen phthalate as a spraying reagent. The sugar moiety was thus confirmed to be glucose. The chemical and spectroscopic evidence thus confirmed substance F as phegopolin[68] (14) i.e. genkwanin-4'-glucoside.

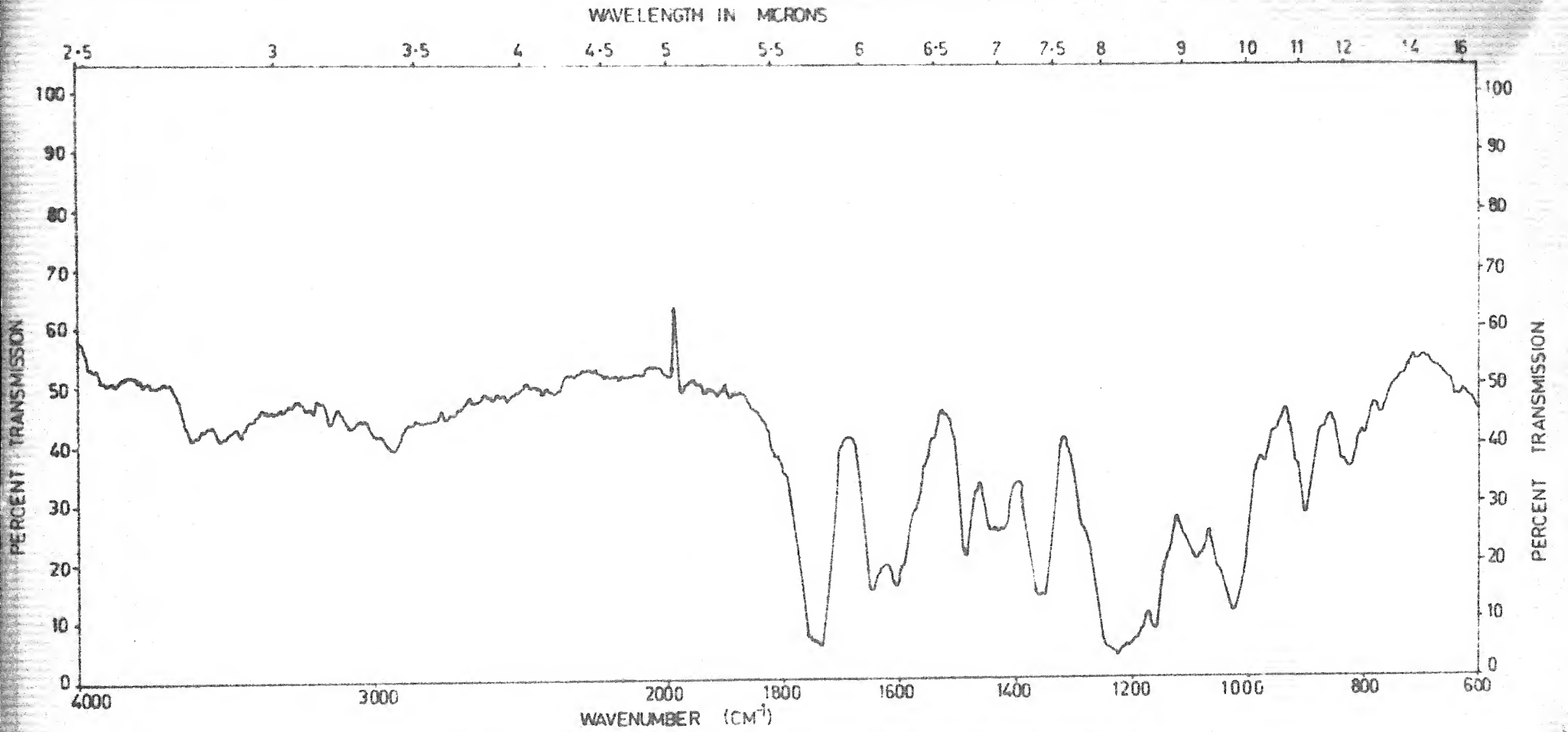


FIG-17 IR SPECTRUM, ACETATE OF SUBSTANCE-F.

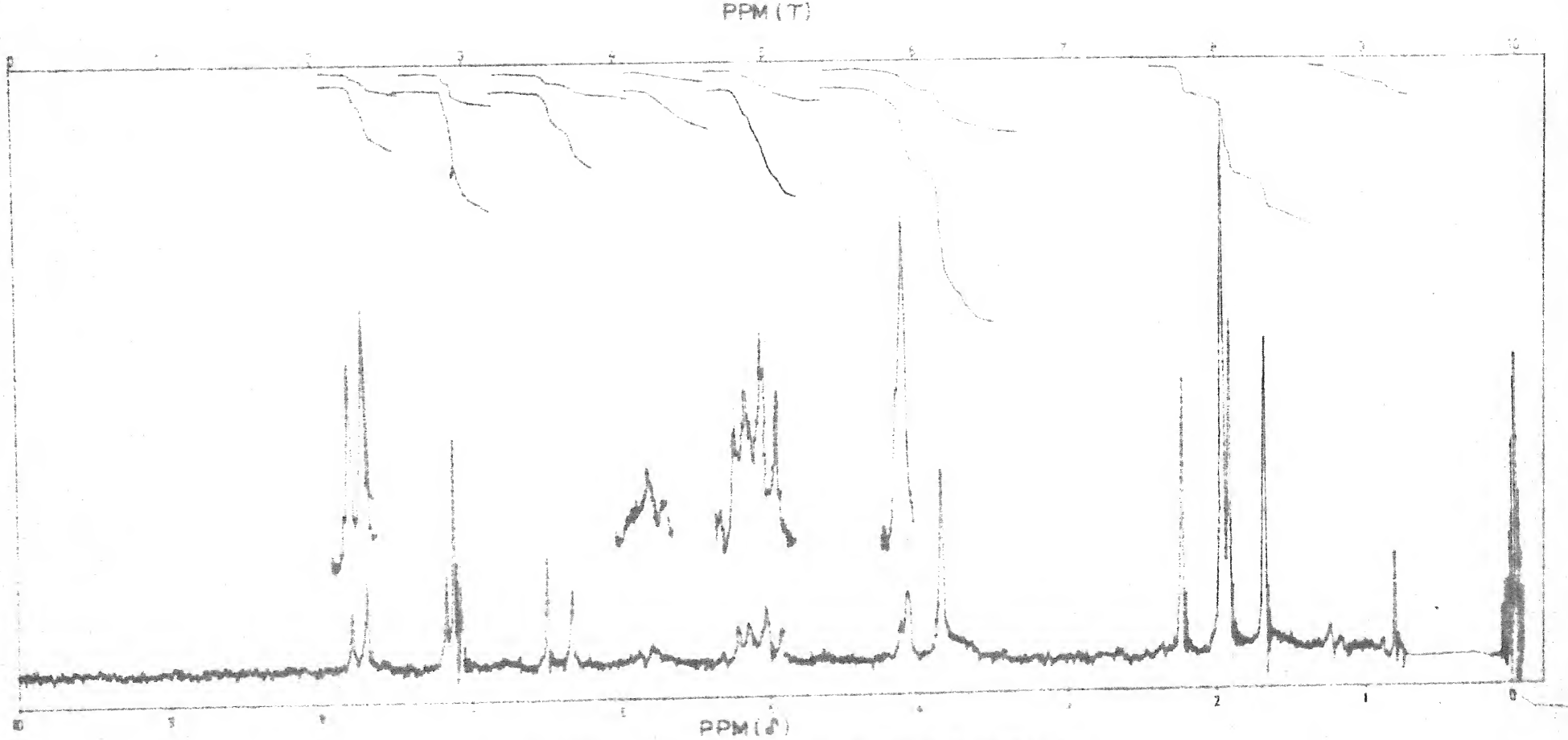


FIG-18 NMR SPECTRUM - ACETATE OF SUBSTANCE-E

Substance G: Crystallised as yellow granules from methanol m.p. 189-90°. It was indicated to be a flavonoid glycoside by positive Shinoda (Mg+HCl) and Fiegel colour tests. It was analysed for $C_{27}H_{30}O_{16}$. The IR spectrum of substance G showed bands at 3400 cm^{-1} for hydroxyl, 1650 cm^{-1} for chelated carbonyl function and 1600, 1555 cm^{-1} for aromatic rings in the molecule.

The UV spectrum[69] (Spectrum No.69) (Fig.19) of substance G exhibited absorption maxima at 259, 266sh, 299sh and 359 nm characteristic of flavonoids. A 12 nm bathochromic shift of band II with addition of sodium acetate was due to the presence of 7 hydroxyl function. A bathochromic shift of 28 nm with sodium acetate in presence of boric acid of band I relative to MeOH and a 31 nm bathochromic shift of band I with $AlCl_3$ relative to $AlCl_3$/HCl was indicative of ortho- dihydroxyl groups. Further a 43 nm bathochromic shift of band Ia (in $AlCl_3$/HCl) to band I (in MeOH) was caused by C-5 hydroxyls and C-3 position seemed to be substitued.

On acidic hydrolysis substance G furnished an aglycone, m.p. 314-15° which analysed for $C_{15}H_{10}O_4$ and supported by mass spectrometry (M^+302). The UV spectrum[69] (spectrum No.65) (Fig.20) of aglycone showed maxima at 370, 301sh, 269 and 255 nm similar to flavonols. A 6 nm bathochromic shift of band II was caused by C-7 hydroxy function in presence of sodium acetate, a bathochromic shift of 85 nm with $AlCl_3$ and reduction of the shift in

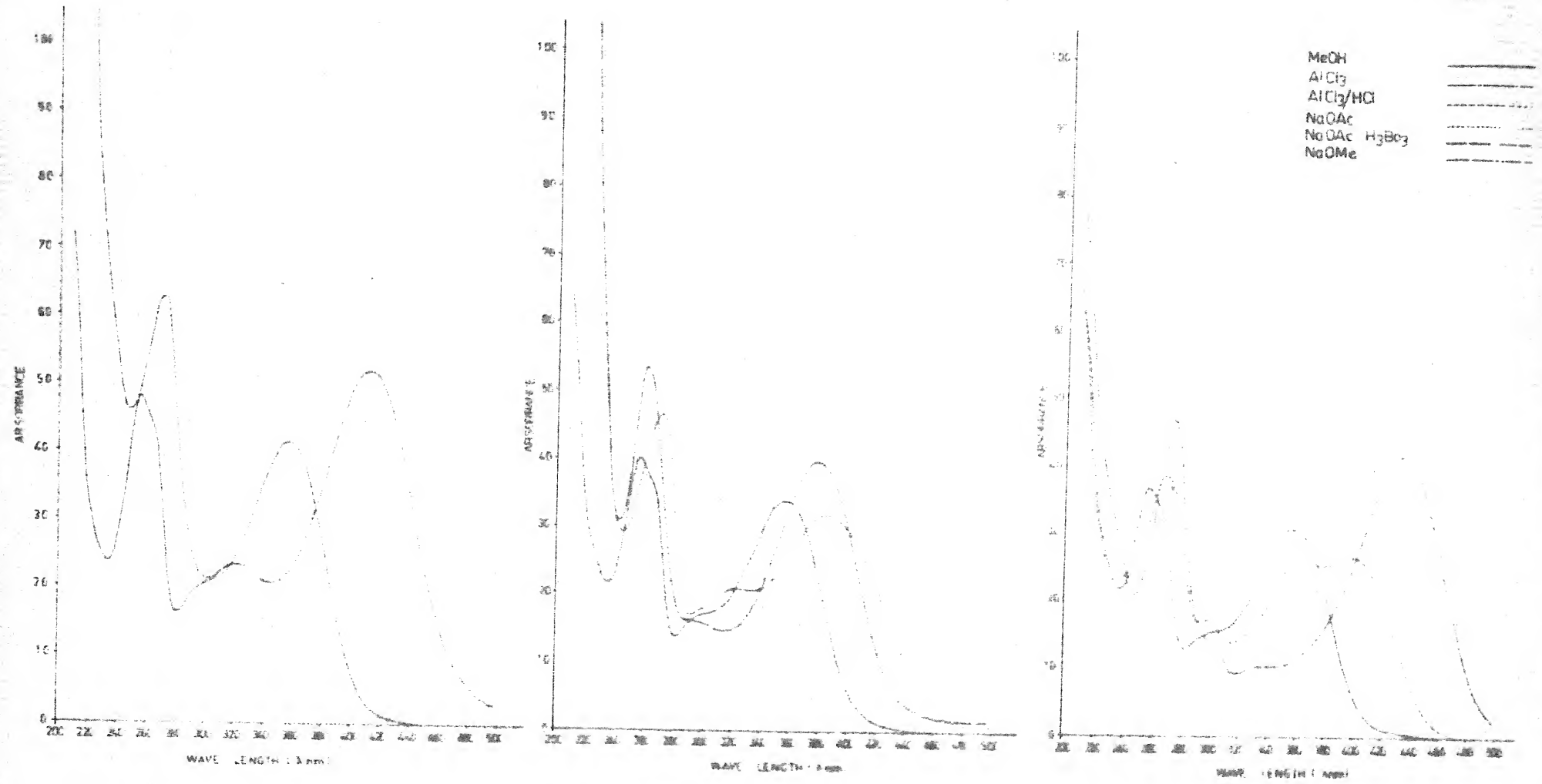


FIG-19 UV SPECTRUM OF SUBSTANCE-G

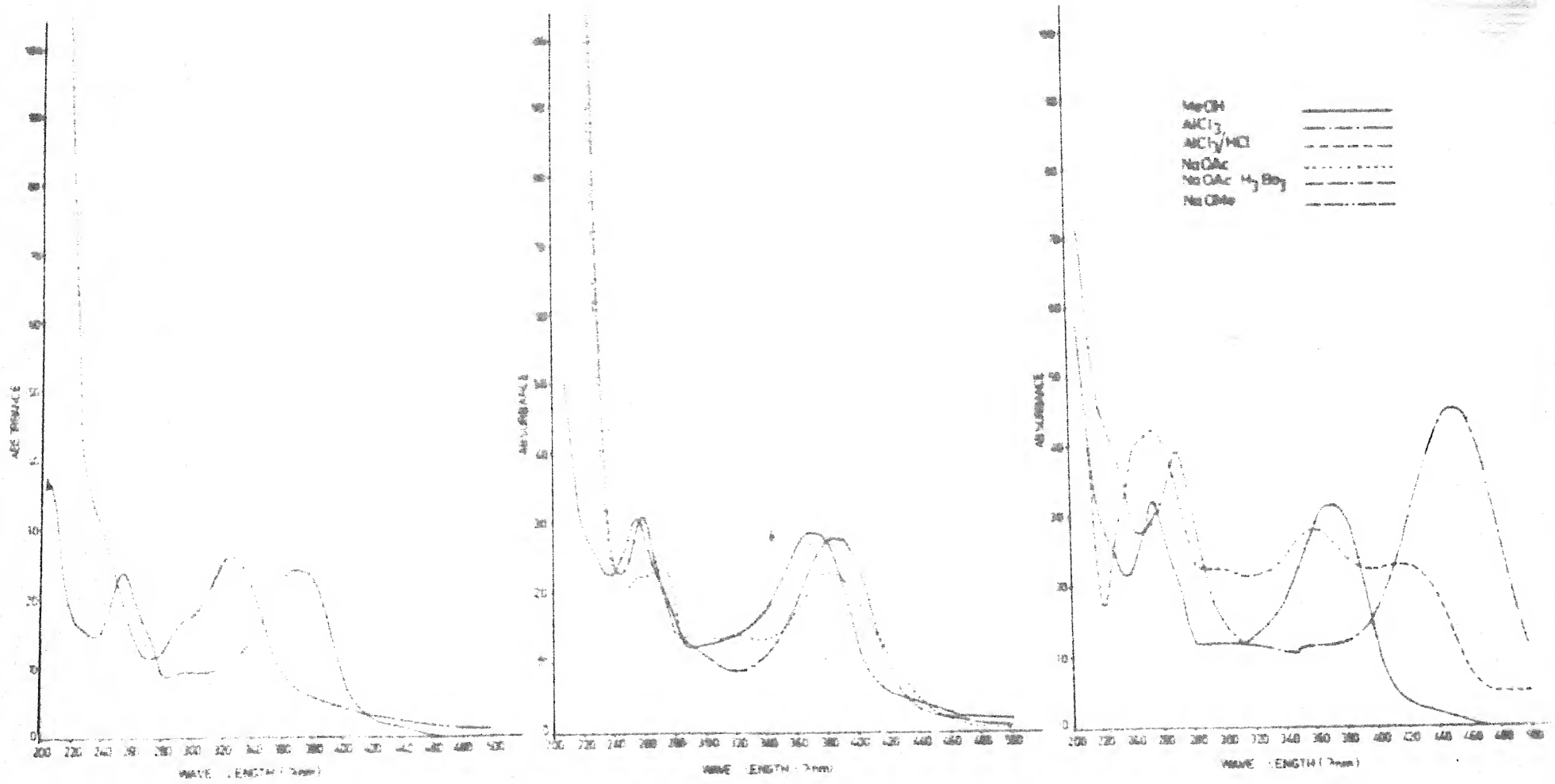


FIG-20 UV SPECTRUM - AGLYCONE OF SUBSTANCE-G

$AlCl_3$/HCl to 58 nm were indicative of C-3 and C-5 hydroxyls alongwith an ortho-dihydroxyl function in the molecule. In the NMR spectrum (Fig.21) the aromatic protons of ring A appeared at δ 6.16 and 6.38 (2H , AB system, J= 2.5 Hz, H-6 and H-8) and ring B at 6.86 (1H, J= 9.0 Hz, H-5'), δ7.59 (1H, J= 9 Hz,and 2.5 Hz, H-6') and δ7.71 (1H, J= 2.5 Hz, H-2'). The mass spectrum (Fig.22) of the aglycone showed molecular ion at m/z 302 and fragment ions at 301 $(M-H)^+$, 152, 153 and 137. The fragmentation pattern (Scheme-2) indicated the presence of hydroxyls at C-5, C-7, C-3' and C-4' in the molecule. The physical data of aglycone was indentical to that of quercetin[70] (17) which was confirmed by co-TLC and m.m.p. The aqueous fraction was neutralised and the sugar moiety was identified as glucose and rhamnose by paper chromatography. The substance G was thus identified as rutin[71] (16) and confirmed by direct comparison with an authentic sample.

Substance H: m.p.222-225°. Crystallised from methanol. It was indicated to be a flavone glycoside by positive Shinoda test and Fiegel colour tests. The IR spectrum (Fig.23) of substance H showed a sharp hydroxyl band at 3400 cm^{-1}, a chelated carbonyl group at 1656 cm^{-1}, aromatic rings at 1615, 1580 and ether linkage at 1210 cm^{-1} in the molecule. The UV spectrum[69](Spectrum No.66) of substance H showed absorption maxima at 256, 272 and 347 nm. A bathochromic shift of 28 nm in band I with addition of sodium acetate in presence of boric acid relative to

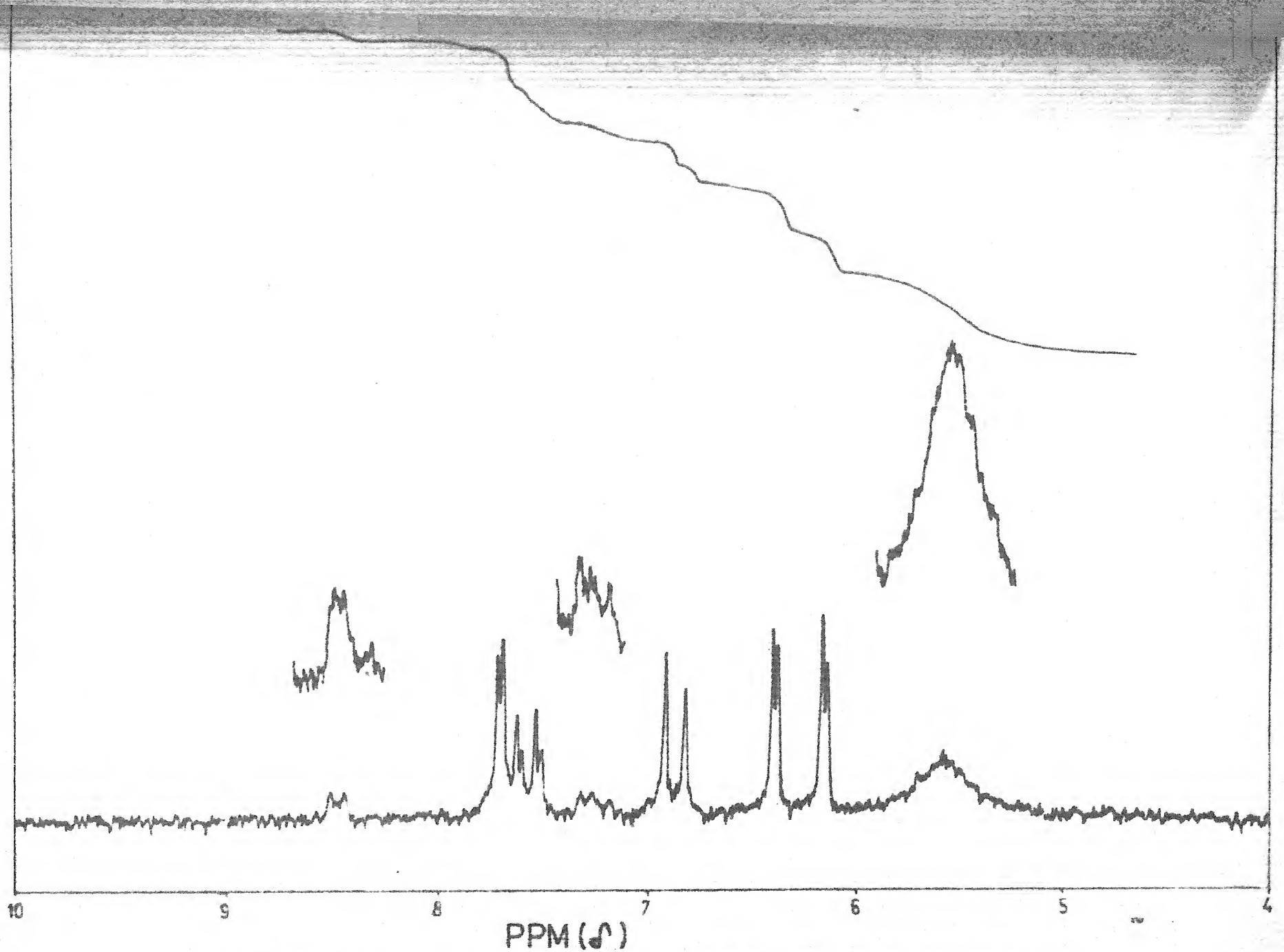


FIG-21 NMR SPECTRUM- AGLYCONE OF SUBSTANCE-G.

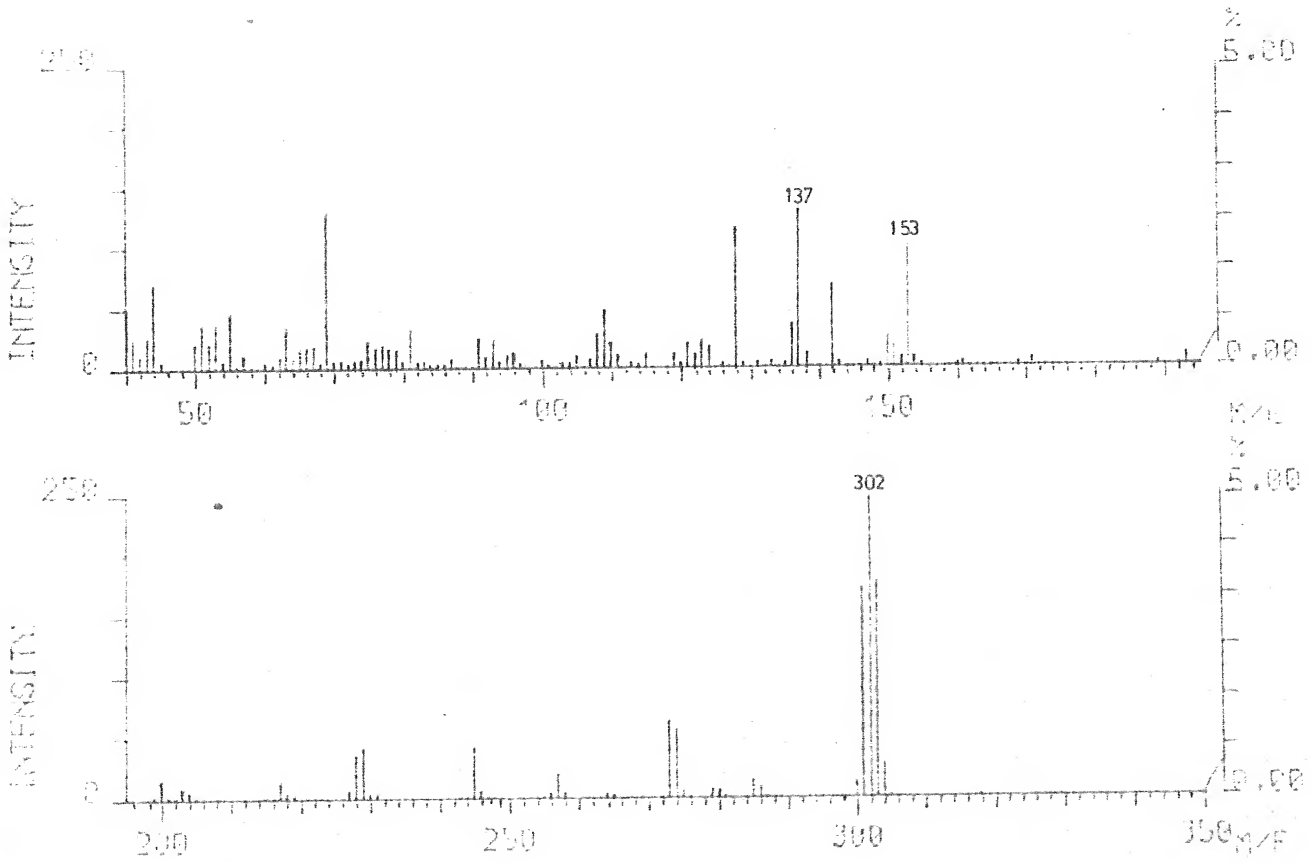


FIG-22 EI MASS SPECTRUM
AGLYCONE OF SUBSTANCE-G.

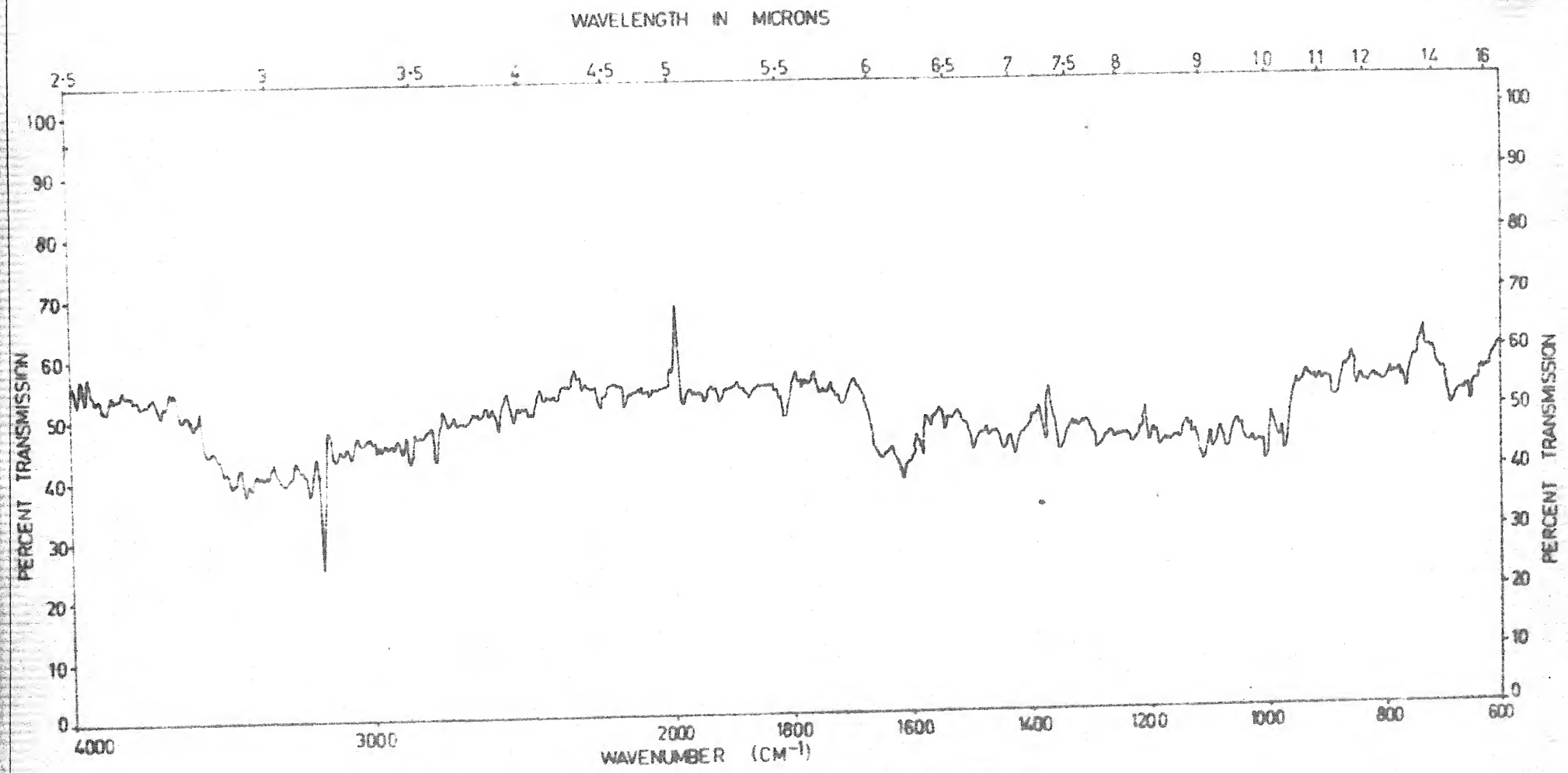


FIG-23 IR SPECTRUM OF SUBSTANCE-H

methanol spectrum and also a bathochromic shift of 31 nm in band I with $AlCl_3$ relative to $AlCl_3$/HCl spectrum suggested the presence of ortho-dihydroxyl groups in the flavone nucleus. Further, a 53 nm bathochromic shift observed in band Ia (in $AlCl_3$/HCl) relative to band I (in MeOH) indicated the presence of free hydroxyl groups at C-5 and C-3 positions. Since no shift appeared in band II with sodium acetate relative to MeOH, there is no free hydroxyl group at C-7 position. A bathochromic shift of 63 nm in band I with addition of sodium methoxide further confirmed the presence of free C-4' hydroxyl group in the molecule. Its FD mass spectrum (Fig.24) showed prominent peaks at m/z 449 $(M+H)^+$ and 471 $(M+Na)^+$ and thus the molecular weight was confirmed as 448. The presence of a small peak at m/z 303 presumably formed by the loss of sugar unit indicates that the sugar unit has a molecular weight of 164. The EIMS of substance H showed major peaks at m/z 302 $(M-146)^+$, 153, 152 and 137. The fragmentation pattern (Scheme-3) alongwith the UV data indicated the presence of hydroxyls at C-5, C-7, C-3' and C-4' positions in the molecule. Hence it appears that the sugar is attached to the C-7 position.

Acid hydrolysis of substance H with 6% methanolic HCl afforded an aglycone which was confirmed as quercetin by co-TLC, paper chromatography and FDMS (M^+, m/z 302). The aqueous portion after neutralisation was found to contain rhamnose by paper chromatography. The FDMS of

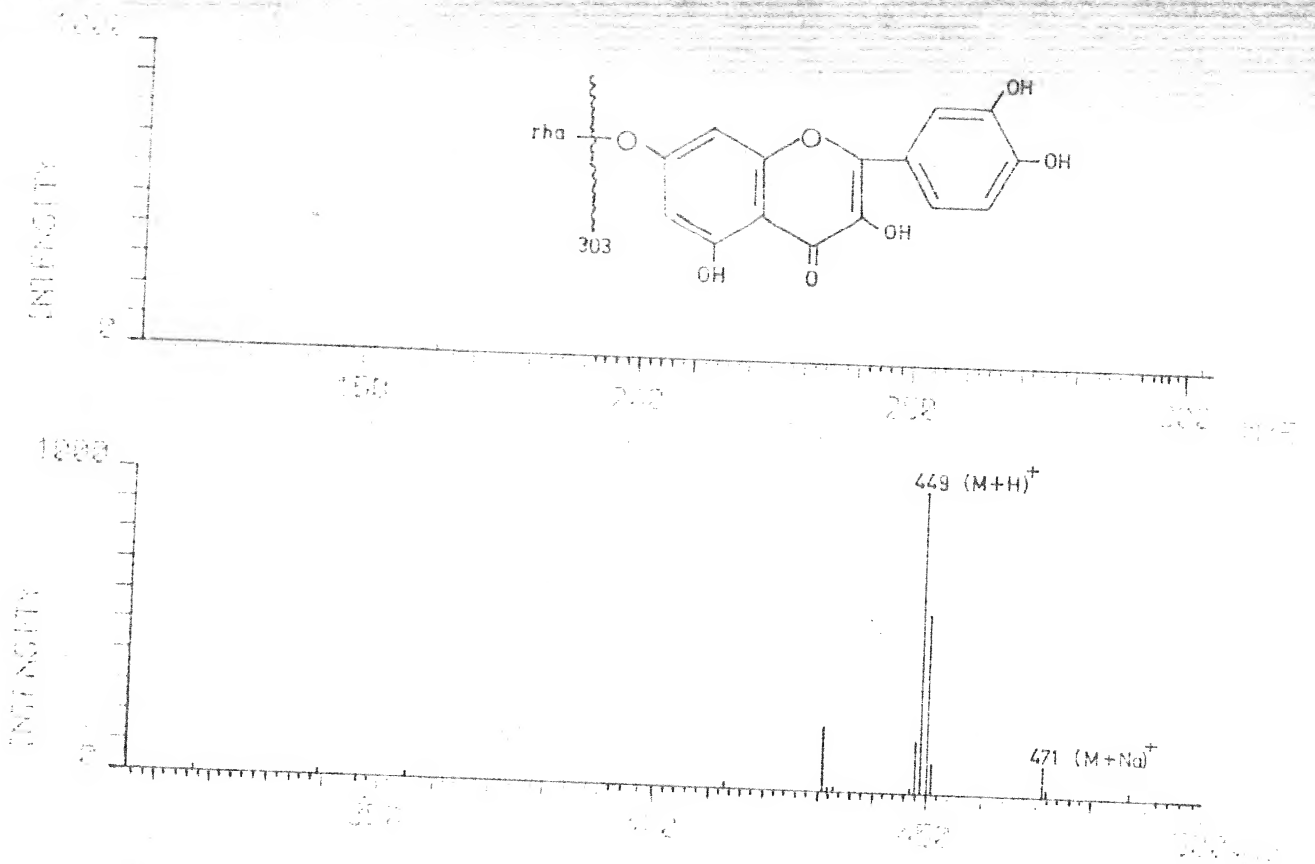


FIG-24 FD MASS SPECTRUM OF SUBSTANCE-H.

the aqueous portion also gave a peak at m/z 165 $(M+H)^+$. The chemical and spectroscopic evidence thus confirmed substance H as quercetin 7-O-rhamnoside[70] (18).

Substance I: It was obtained as a yellow micro-crystalline product. A positive Fiegel test and response to Shinoda test indicated it to be a flavonoid glycoside. The IR spectrum (Fig.25) showed characteristic bands at 3400(O-H), 1650(C=O), 1615, 1580 (C=C) and 1250, (Ar-O-C) cm^{-1}. The UV spectrum (Fig.26) exhibited λ max at 332, 310 and 272 nm. A bathochromic shift of 40 nm in band I without decrease in intensity with addition of sodium methoxide showed the presence of free C-4' hydroxyl group in the molecule. There was no change in the UV spectrum when sodium acetate was added suggesting the absence of free hydroxyl group at C-7 position. As there was no shift in the bands with boric acid buffer, the possibility of ortho-dihydroxyl grouping in the molecule is ruled out. A bathochromic shift of 42 nm in band I (in MeOH) to band Ia (in $AlCl_3$/HCl) suggested the presence of free hydroxyl at C-5 position.

The peaks at m/z 433 $(M+H)^+$ and m/z 455 $(M+Na)^+$ observed in the FDMS (Fig.27) of substance I confirmed the molecular weight as 432. A small peak at m/z 301 due to the loss of the sugar unit also could be seen in the spectrum. These observation suggested that the sugar unit in substance I has a molecular weight of 150 and the aglycone has a molecular weight of 300. The electron impact mass spectrum of the compound showed major peaks at

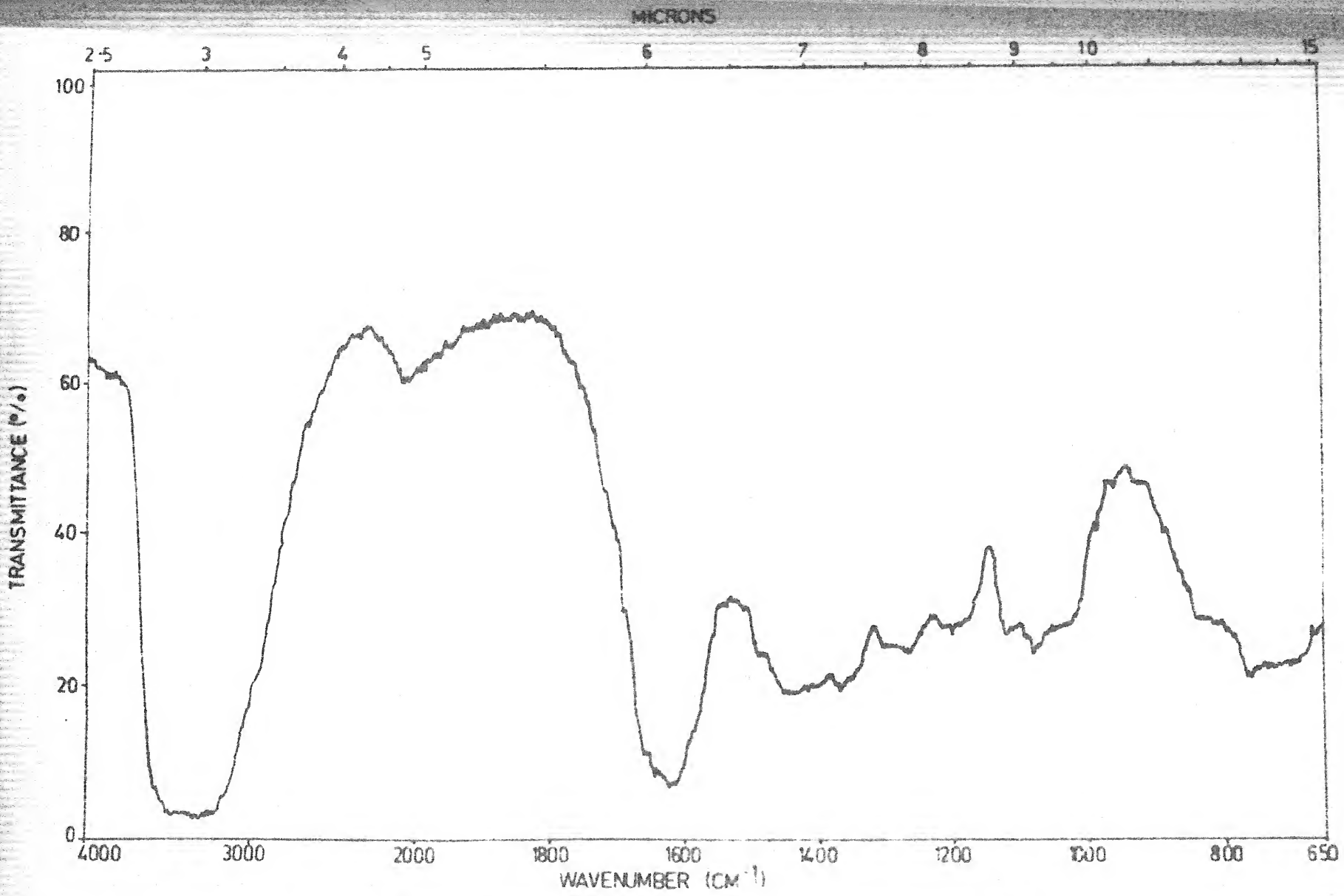


FIG-25 IR SPECIRUM OF SUBSTANCE-I

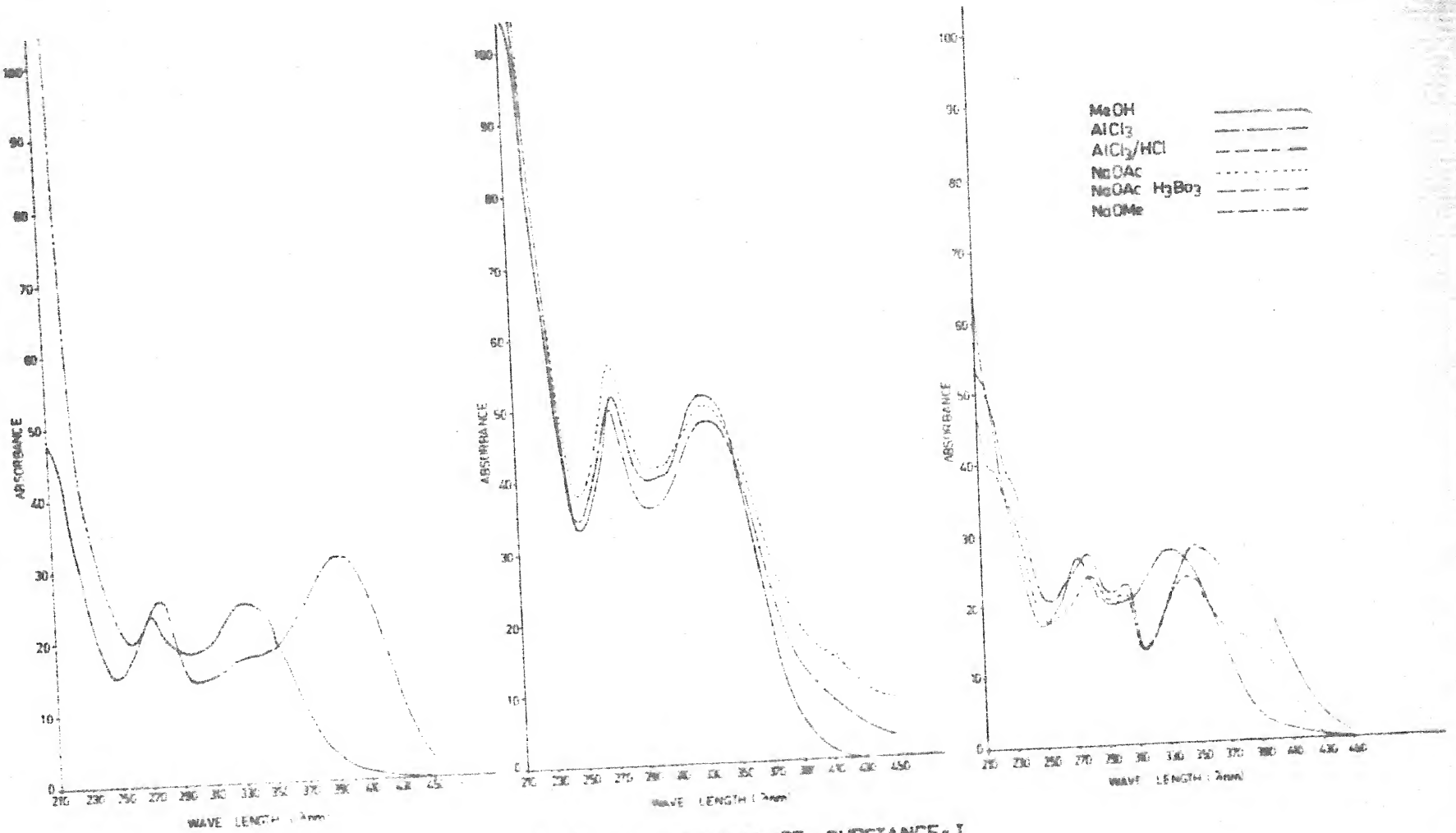


FIG-26 UV SPECTRUM OF SUBSTANCE-I

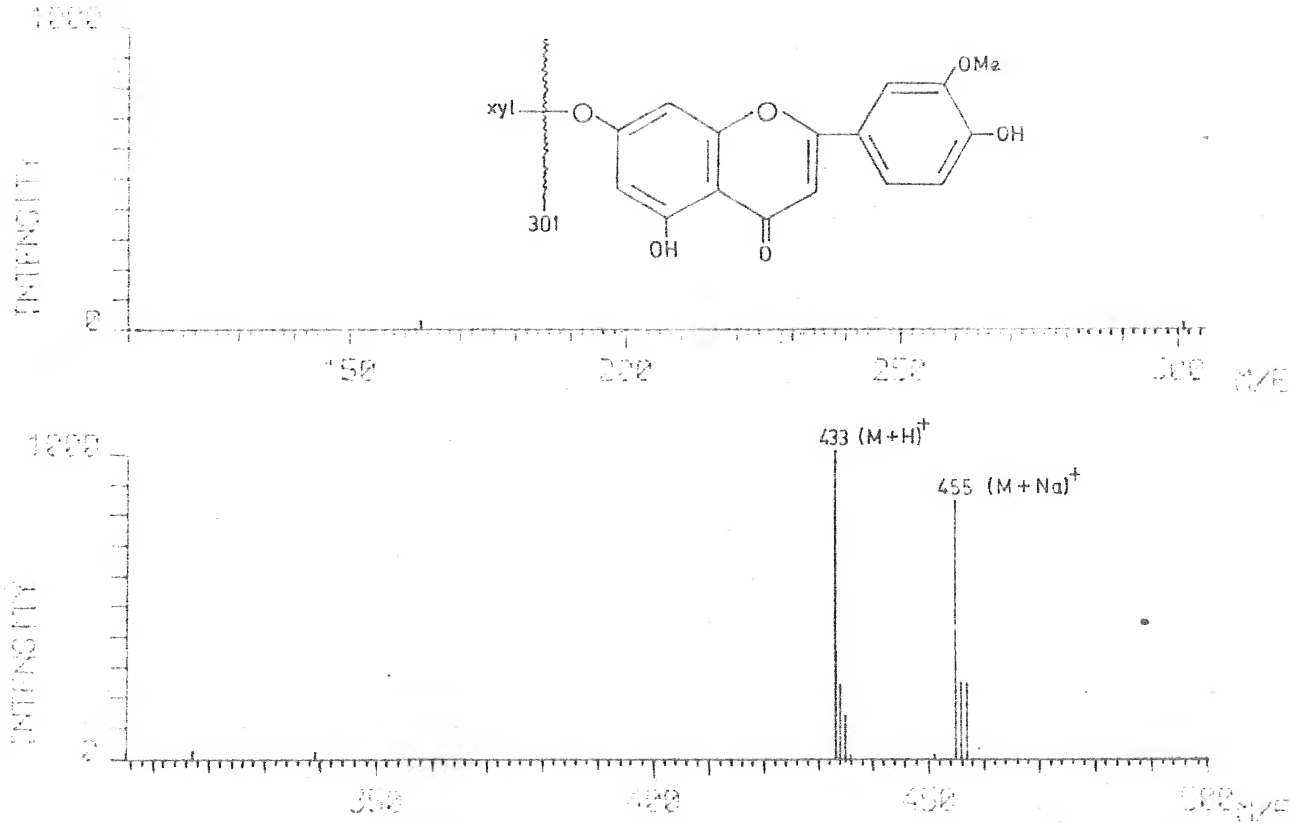


FIG-27 FD MASS SPECTRUM OF SUBSTANCE-I.

m/z 300 $(M-132)^+$, 151, followed by RDA fragments at m/z 153, 152 and 148. The major fragment peak at m/z 151 suggests the presence of $-OCH_3$ in the B ring (Scheme-4).

On acid hydrolysis of substance I, an aglycone was obtained. The UV spectrum (Fig.28) of the aglycone exhibited absorption maxima at 347, 330, 269, 249 and 241 nm. A 45 nm bathochromic shift observed in band I with NaOMe, without decrease in intensity suggested the presence of free C-4' hydroxyl group. Further, a bathochromic shift of 30 nm in band II with sodium acetate relative to MeOH indicated the presence of C-7 hydroxyl group and a bathochromic shift of 39 nm in band I with $AlCl_3$/HCl relative to MeOH showed the presence of C-5 hydroxyl group. The UV spectrum[69](Spectrum No.40) of aglycone was super-imposable to that of chrysoeriol[72] (20). The FDMS of the aglycone showed a peak at m/z 300. Its identity as chrysoeriol was further confirmed by co-TLC and m.m.p. with an authentic sample.

The sugar moiety was indentified as xylose by paper chromatography and FDMS m/z 151, $(M+H)^+$ of the aqueous fraction.

From the UV spectrum of the aglycone it is clear that the sugar, xylose is attached to the C-7 position of the flavonoid nucleus. This is also supported by the mass spectral fragment pattern. The substance I was thus identified as chrysoeriol-7-O-xyloside [73](19).

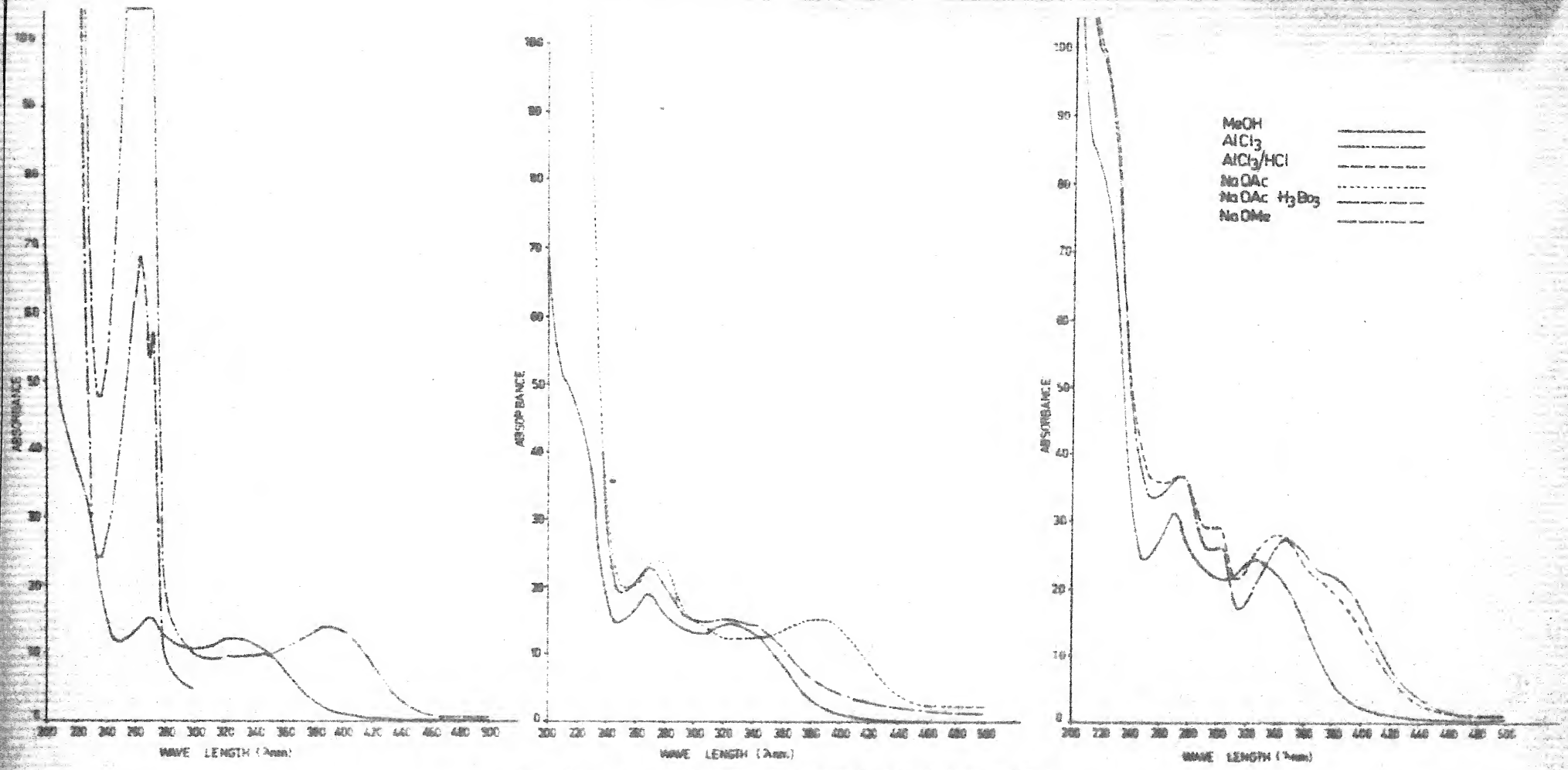


FIG-28 UV SPECTRUM AGLYCONE OF SUBSTANCE-I

Substance J: m.p. 186-187°, (lit.m.p. 186-88°)[74], $(\alpha)_D^{22°}$ +67 (H_2O), (lit. $(\alpha)_D^{25°}$ + 65.5°, (H_2O)). It was analysed for $C_7H_{14}O_6$ and confirmed by FDMS $(M+H)^+$, m/z 195 (Fig.29). The IR absorption (Fig.30) displayed a broad band at 3380 cm^{-1} and a sharp peak at 3330 and 1080 cm^{-1} revealed the presence of a hydroxy function. It is sparingly soluble in methanol, ethanol and acetone but freely soluble in water. It failed to decolourise Fehling's solution,[75] indicating it to be a non reducing sugar. Acetylation of substance J furnished a penta-acetate, m.p. 98°. Its IR spectrum showed the absence of hydroxyl function and the new bands at 1730 and 1210 cm^{-1} appeared for the acetoxy function. It had an Rf value of 0.49 on paper chromatogram developed in acetone-water (85:15, v/v)[76]. The substance J was thus identified as pinitol[60,77] (21) and its physico-chemical data corresponded to the reported values in literature.

Substance K: m.p. 237°(dec). Crystallised from methanol. It developed pale yellow colour with Shinoda test and intense yellow on exposing to ammonia vapours. A positive Fiegel test indicated it to be a glycoside. The IR spectrum (Fig.31) showed bands at 3380 cm^{-1} for hydroxyl group, 1650 cm^{-1} for chelated carbonyl system, 1610, 1580 cm^{-1} for aromatic nucleus and 1250 and 1060 cm^{-1} ether linkage (Ar-O-C). The UV spectrum (Fig.32) showed absorption maxima at 334, 302 and 272 nm. A bathochromic shift of 8 nm in band II in the presence of sodium acetate

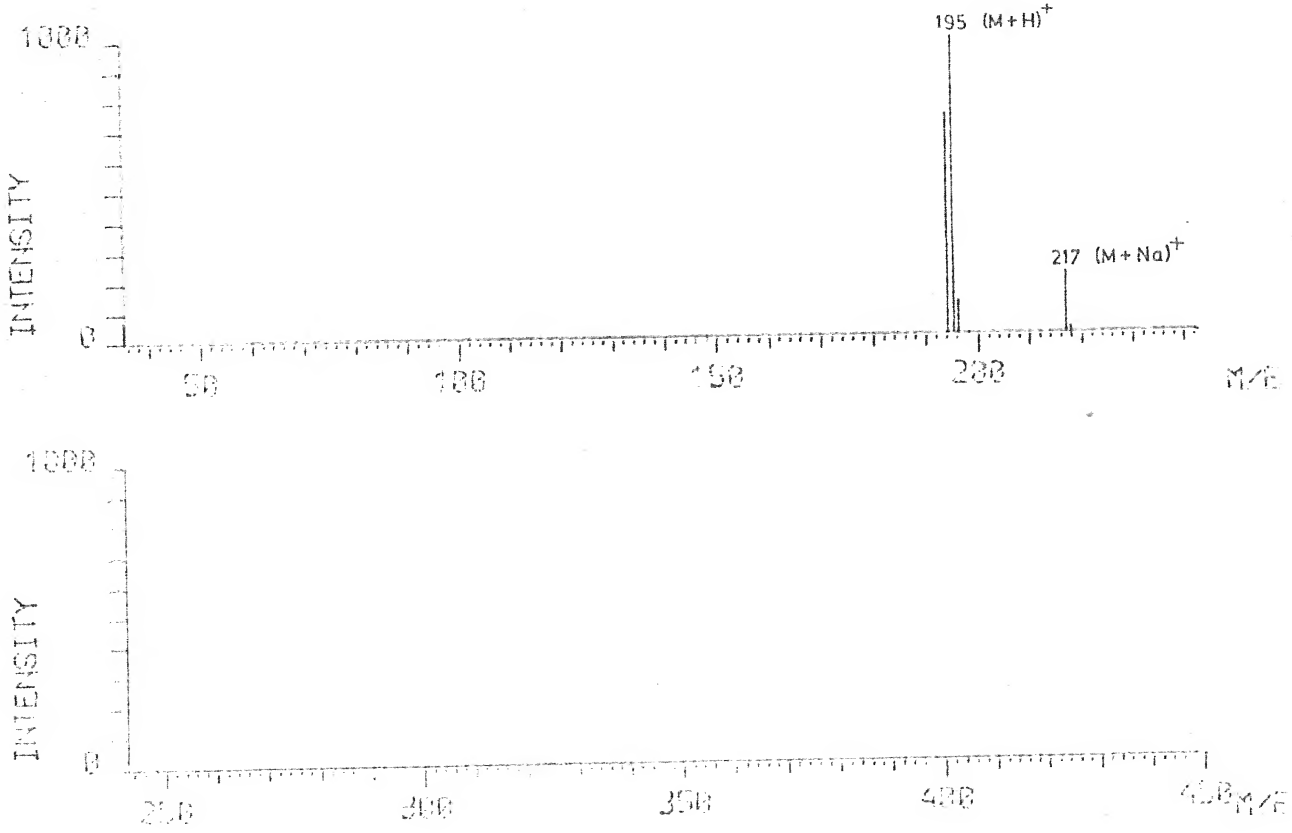


FIG-29 FD MASS SPECTRUM OF SUBSTANCE-J.

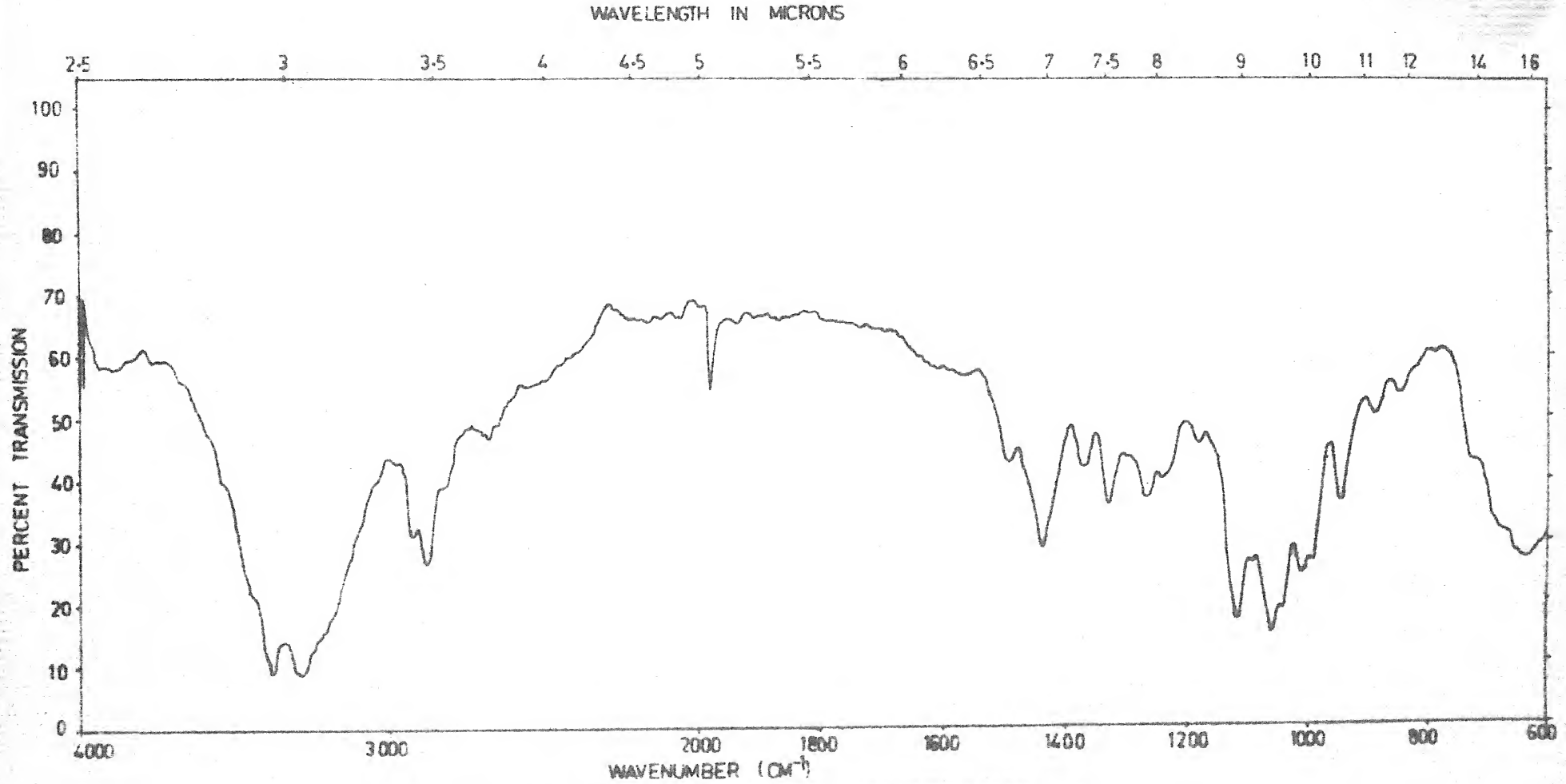


FIG-30 IR SPECTRUM OF SUBSTANCE-J

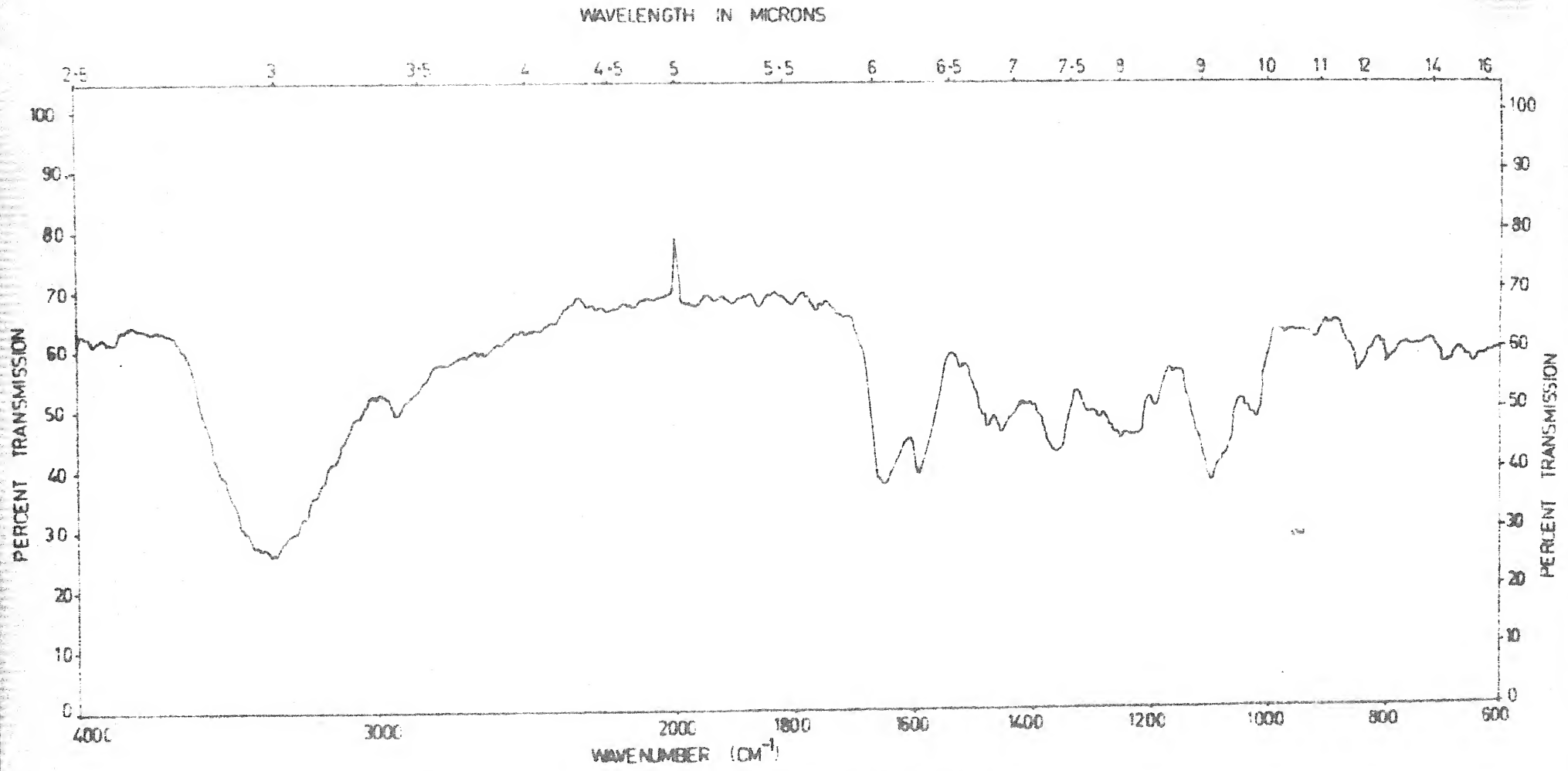


FIG-31 IR SPECTRUM OF SUBSTANCE-K

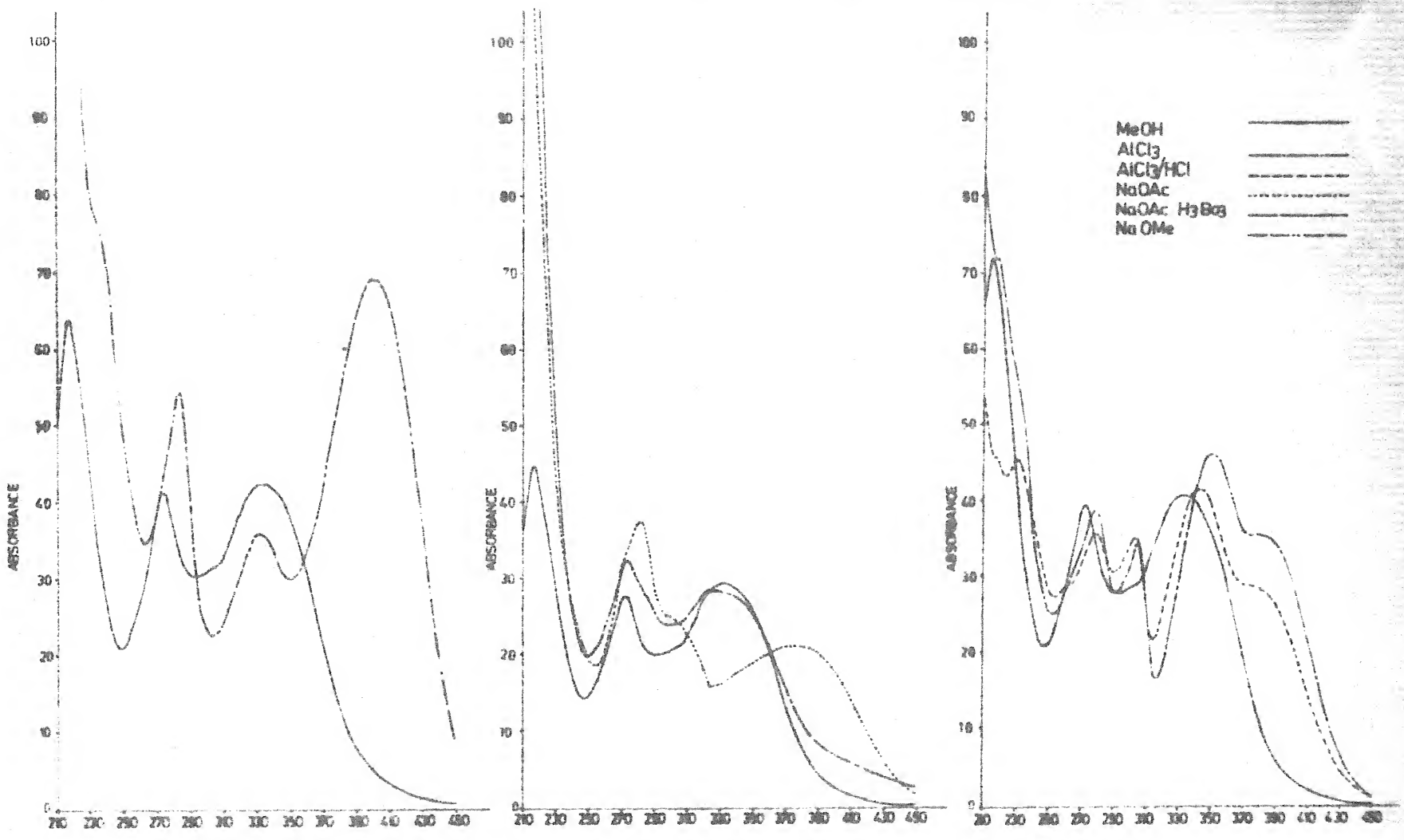


FIG-32 UV SPECTRUM OF SUBSTANCE-K

indicated the presence of free 7-hydroxyl group in the flavone nucleus. Since there was no bathochromic shift with boric acid-buffer the possibility of ortho-dihydroxyl grouping in the molecule is ruled out. A bathochromic shift of 45 nm in band Ia with $AlCl_3$/HCl relative to band I in MeOH indicated the presence of free hydroxy groups at C-5 and substitution at C-3. A bathochromic shift of 66 nm in band I without decrease in the intensity with addition of sodium methoxide showed the presence of free hydroxyl group at C-4' in the molecule. The NMR spectrum showed the aromatic protons as singlet at δ 6.28 and 6.72 (2H, AB system, J = 2.5 Hz, ring A protons H-6 and H-8) and δ 7.80 and 6.80 (4H, A_2B_2 system, J = 10.0 Hz, ring B protons, H-2', 6', and H-3',5'). The rhamnose methyl appeared as a complex multiplet at δ 1.20. A broad signal at δ 2.99 - 4.0 represents nine sugar protons. Two anomeric protons of both the sugars appeared between δ 4.2 - 4.6. The FD mass spectrum (Fig.33) showed prominent peaks at m/z 565 $(M+H)^+$, m/z 587 $(M+Na)^+$ and thus the molecular weight was confirmed as 564. The peaks at m/z 455, 433 and 419 corresponded to the loss of 132 from $(M+Na)^+$ and $(M+H)^+$ and 146 from $(M+H)^+$ respectively, suggesting the presence of two sugar units in the molecule having molecular weight 150 and 164. The EIMS of substance K showed major peaks at m/z 286 $(M-278)^+$, 285, 153, 152 and 121.

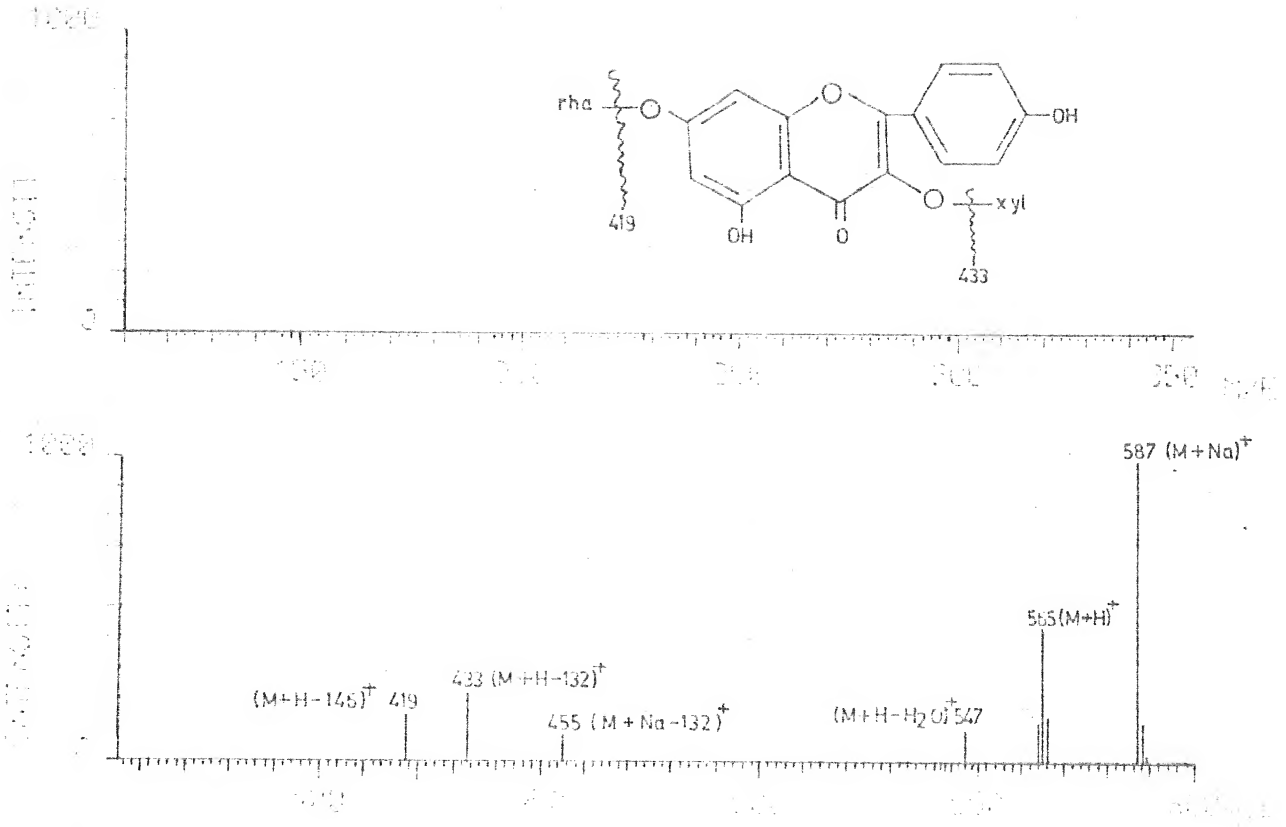


FIG-33 FD MASS SPECTRUM OF SUBSTANCE-K.

Acid hydrolysis of substance K afforded an aglycone. The UV spectrum [40] (Fig.34) of the aglycone showed absorption maxima at 327, 298, 272 and 253 nm. A bathochromic shift of 67 nm in band I without decrease in intensity with addition of sodium methoxide suggested the presence of free hydroxyl at C-4' position. Further, a bathochromic shift of 8 nm in band II with sodium acetate relative to MeOH showed the presence of free hydroxyl at C-7 position. A bathochromic shift of 57 nm in band Ia with $AlCl_3$ to band I of MeOH suggested the presence of 3, 5 dihydroxyl pattern in the flavone nucleus. The UV spectrum [69] (Spectrum No.58) of aglycone was super-imposable to that of Kaempferol [78] (23). Its identity as Kaempferol was confirmed by co-TLC and m.m.p. The aqueous layer after neutralisation showed the presence of two sugars. The presence of xylose and rhamnose was confirmed by paper chromatography and FDMS. The UV spectrum of aglycone clearly indicated that C-3 and C-7 positions are substituted in the glycoside. This was also supported by the mass spectral fragmentation pattern (Scheme-5).

Based on the above data it appears that substance K is a diglycoside of Kaempferol. It could either be kaempferol -3-O-xyloside-7-O- rhamnoside or Kaempferol-7-O-xyloside -3-O- rhamnoside. It has been shown earlier by Mabry and co-workers [79] that in case of quercetin 3-O-rhamnoside the rhamnose CH_3 appears as a doublet at δ 0.85 and in the case of quercetin 7-O-rhamnoside-3-O-glucoside the rhamnose CH_3 appears as a complex

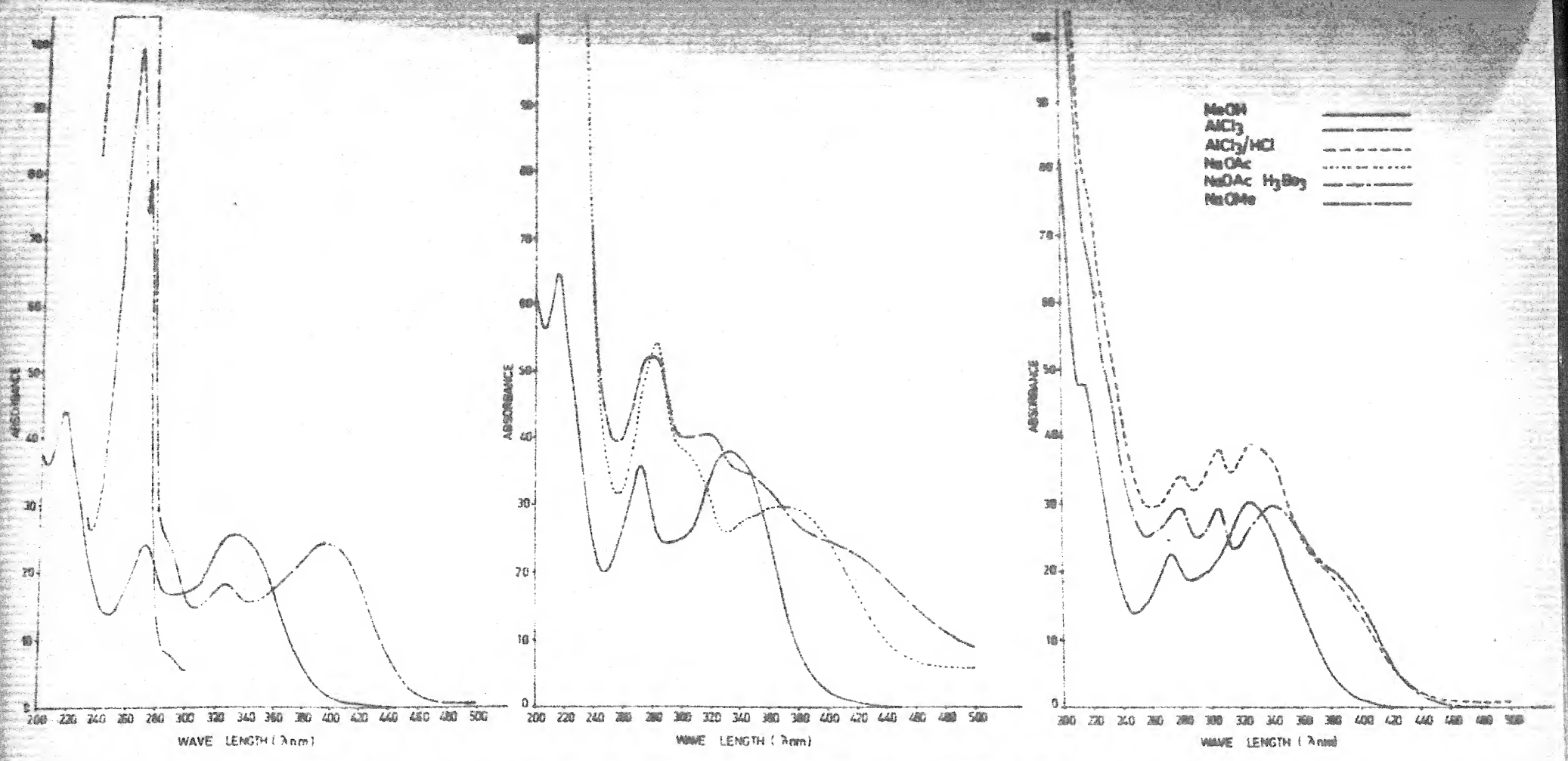


FIG-34 UV SPECTRUM - AGLYCONE OF SUBSTANCE-K

signal at δ 1.2. This effect may be due to the free rotation possible for a rhamnoside moiety at C-7. Since in the NMR spectrum of substance K the rhamnose CH_3 appeared as a complex multiplet at δ 1.20, rhamnose is attached to C-7 and xylose is attached to C-3 position. Thus the spectral evidence suggested substance K to be Kaempferol-3-O-xyloside-7-O-rhamnoside[80] (22).

Free Sugars: The fractions 39-44 obtained from column chromatography of the butanol soluble fraction of the plant extract were found to be rich in reducing sugars as confirmed by positive Fehling's test.[75] The identity of the free sugars was confirmed by co-chromatography on Whatman paper No.1 with authentic sugar samples using two different solvent system [81-82] i.e. n-butanol-acetic acid-water (4:1:5, v/v, upper) and n-butanol-ethanol-water (4:1:5, v/v, upper) and aniline hydrogen phthalate[83] as a spraying reagent. D-glucose and xylose were confirmed on the basis of identical Rf values with those of known authentic samples.

STRUCTURE OF THE COMPOUNDS

$Me(CH_2)_{25}Me$ (1)

$Me(CH_2)_{26}Me$ (2)

$Me(CH_2)_{27}Me$ (3)

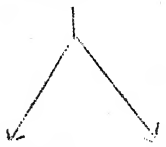
$Me(CH_2)_{28}Me$ (4)

$Me(CH_2)_{29}Me$ (5)

$Me(CH_2)_{30}Me$ (6)

$Me(CH_2)_{31}Me$ (7)

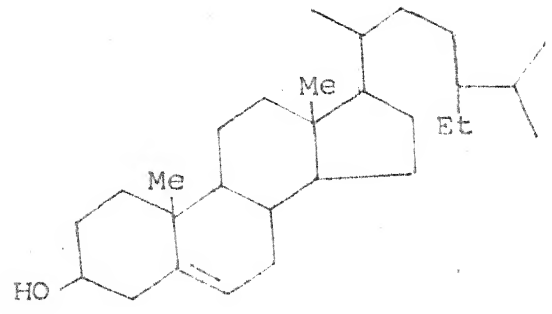
$Me(CH_2)_{25}CO_2(CH_2)_{22}Me$ (8)



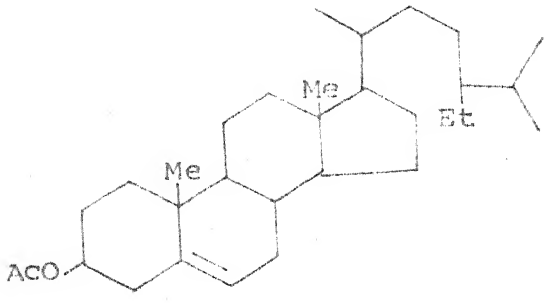
$Me(CH_2)_{21}CH_2OH$ (9)

$Me(CH_2)_{25}CO_2H$ (10)

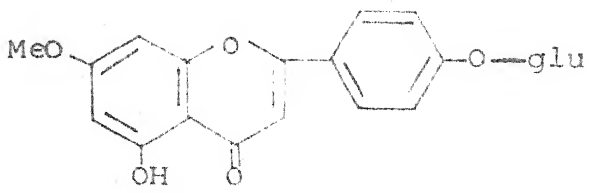
$Me(CH_2)_{28}CH_2OH$ (11)



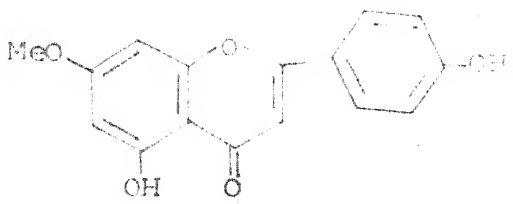
(12)



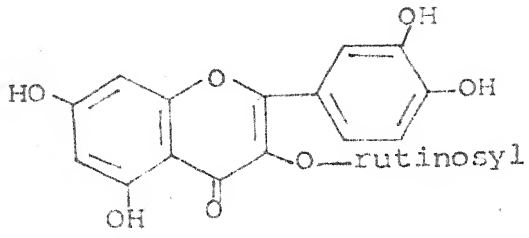
(13)



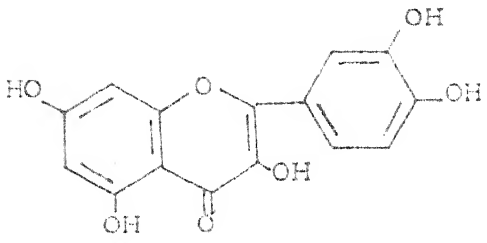
(14)



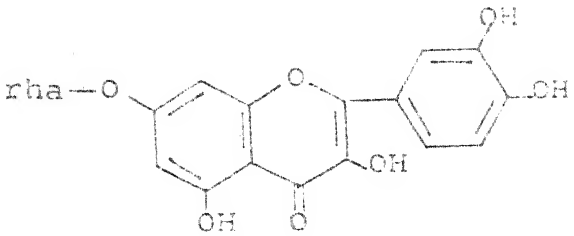
(15)


OH
HO
O
OH
O—rutinosyl
OH
O

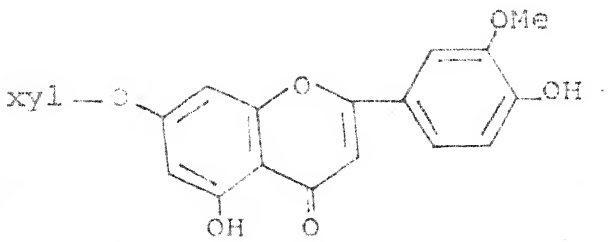
(16)


OH
HO
O
OH
OH
OH
O

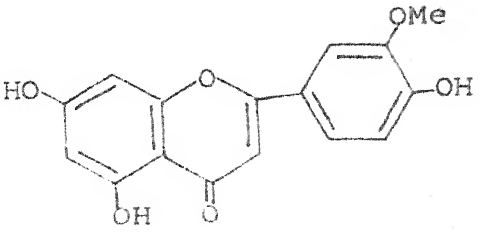
(17)


OH
rha—O
O
OH
OH
OH
O

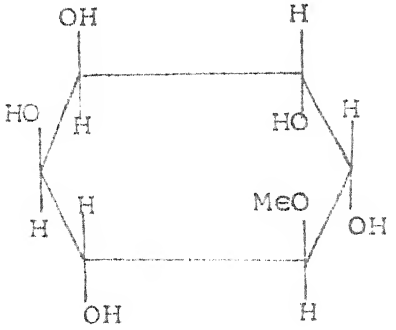
(18)


OMe
xyl—O
O
OH
OH
O

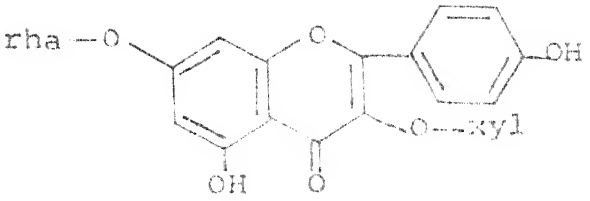
(19)


OMe
HO
O
OH
OH
O

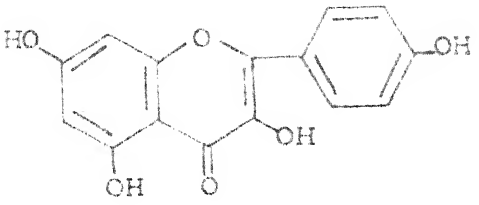
(20)


OH
H
HO
H
HO
H
H
H
MeO
OH
OH
H

(21)

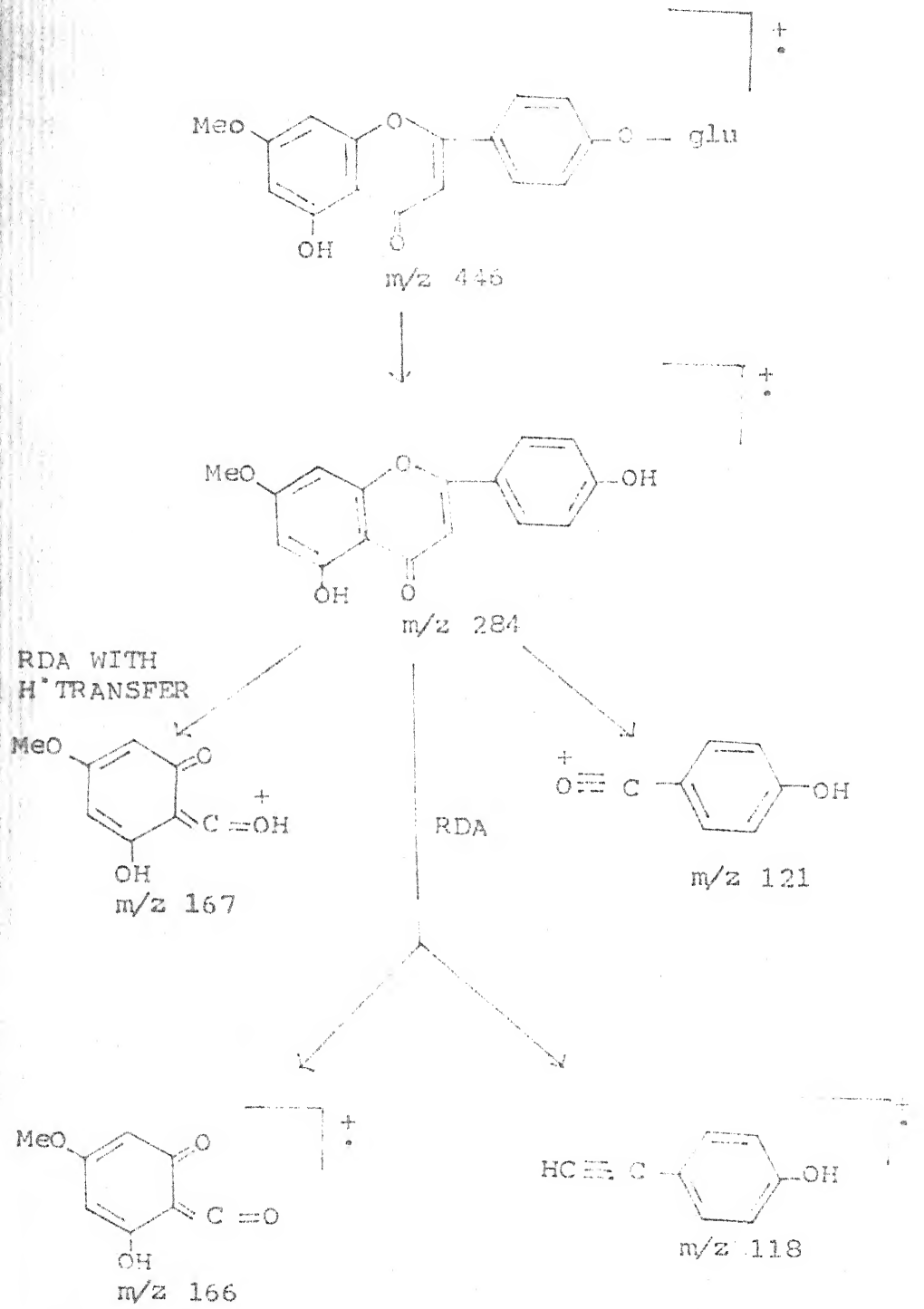

rha —O
O
OH
O—xyl
OH
O

(22)

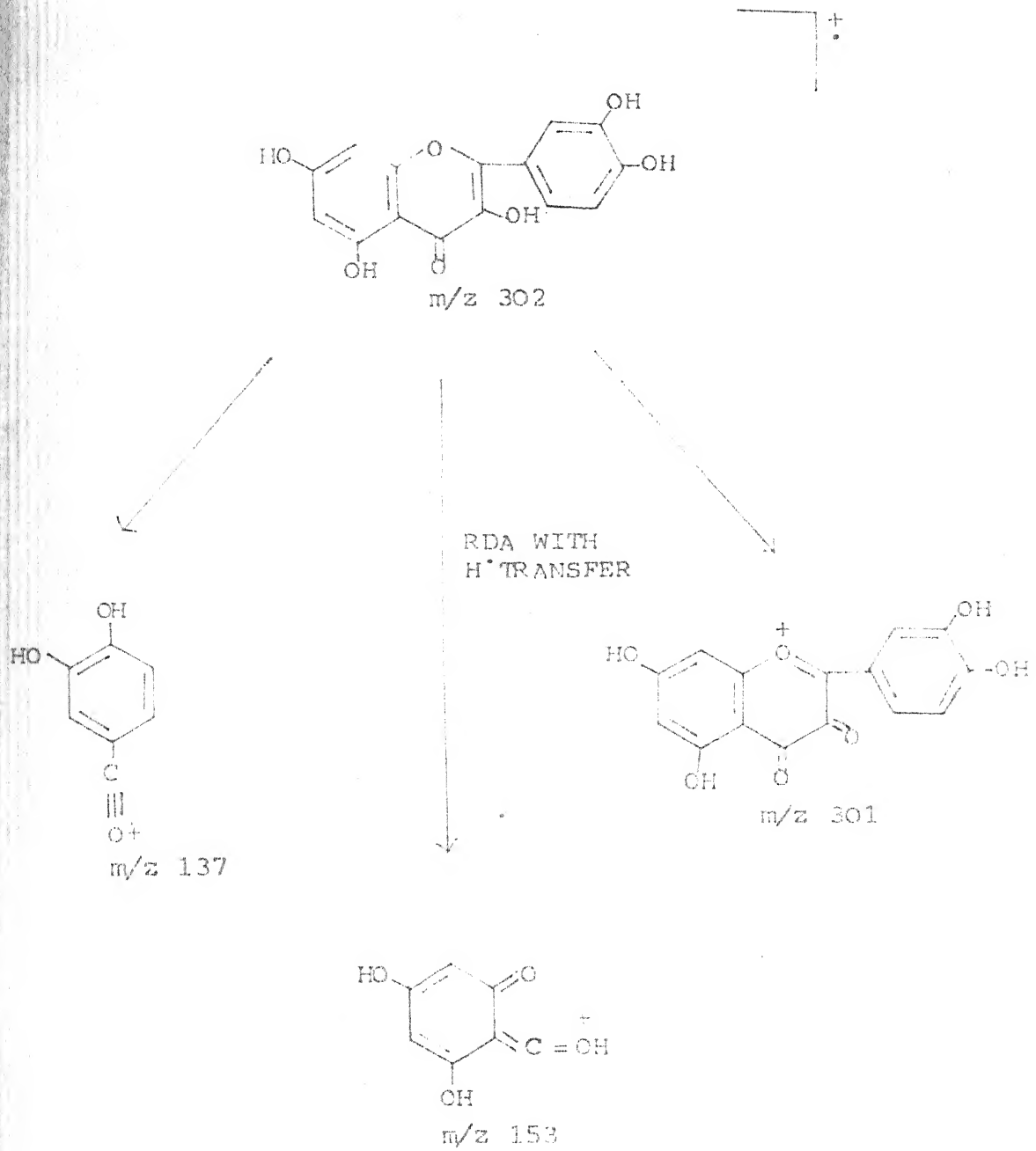

HO
O
OH
OH
OH
O

(23)

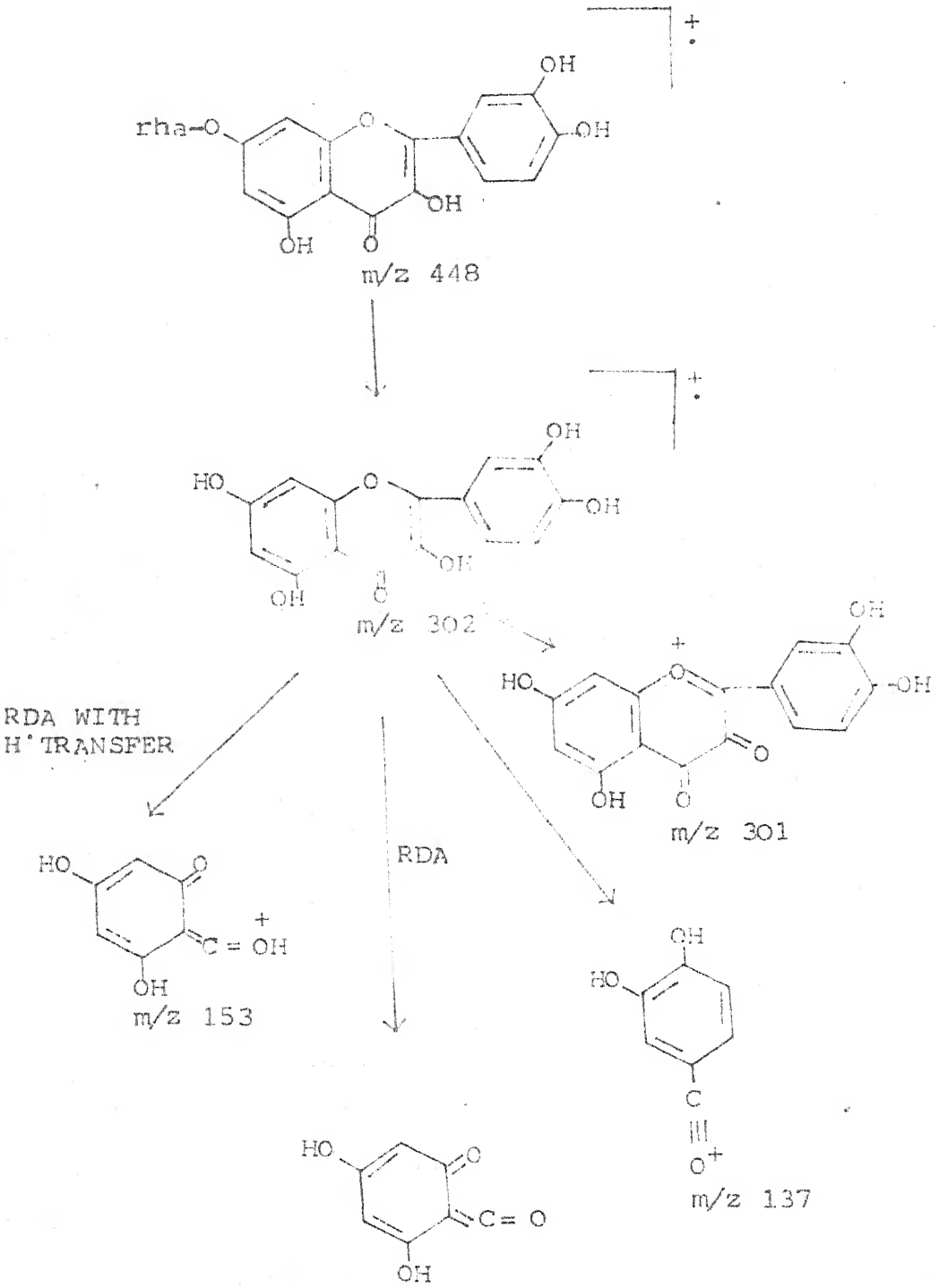
SCHEME - 1 : Mass spectral fragmentation of Phegopolin



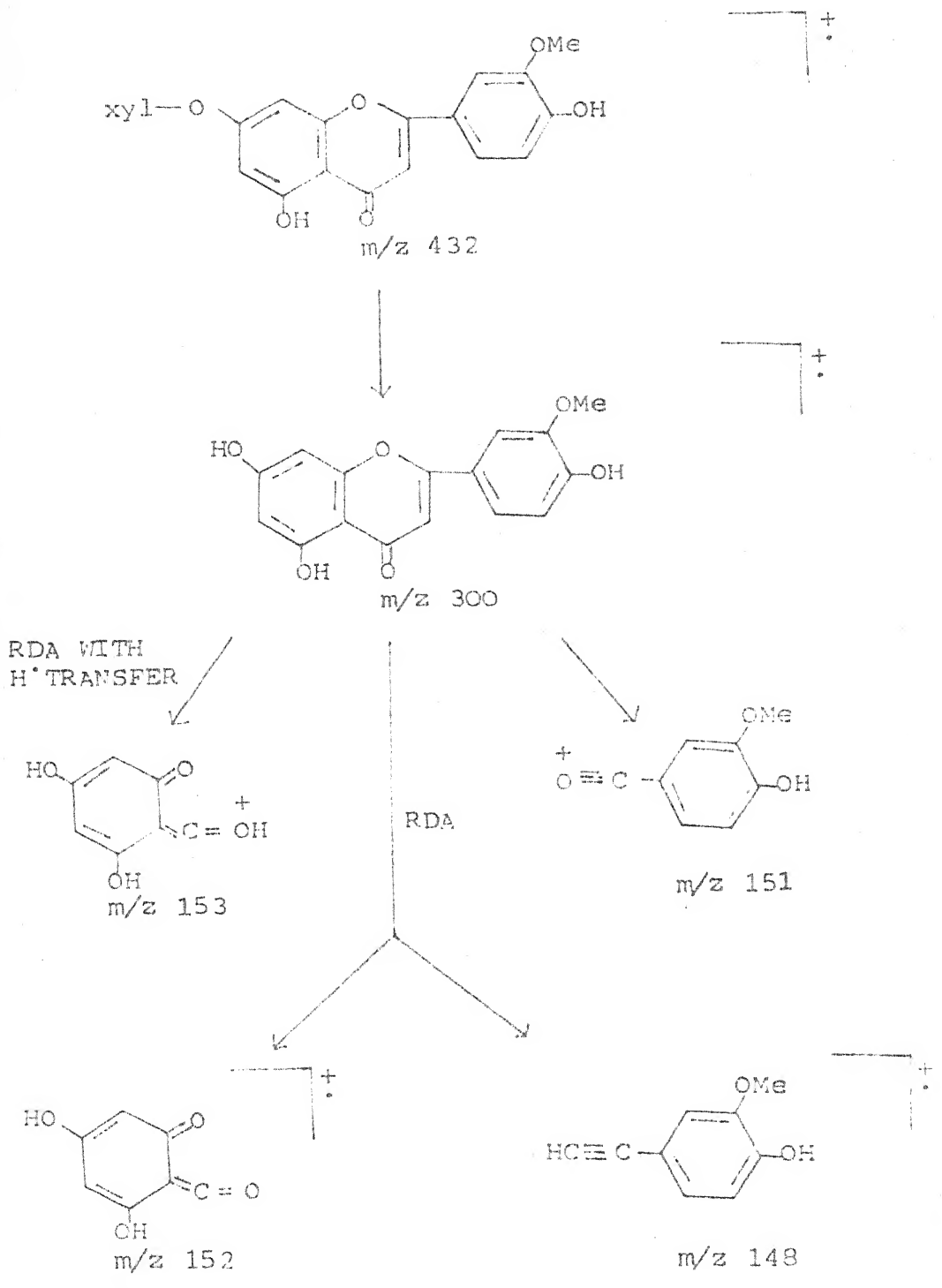
SCHEME - 2: Mass spectral fragmentation of Quercetin



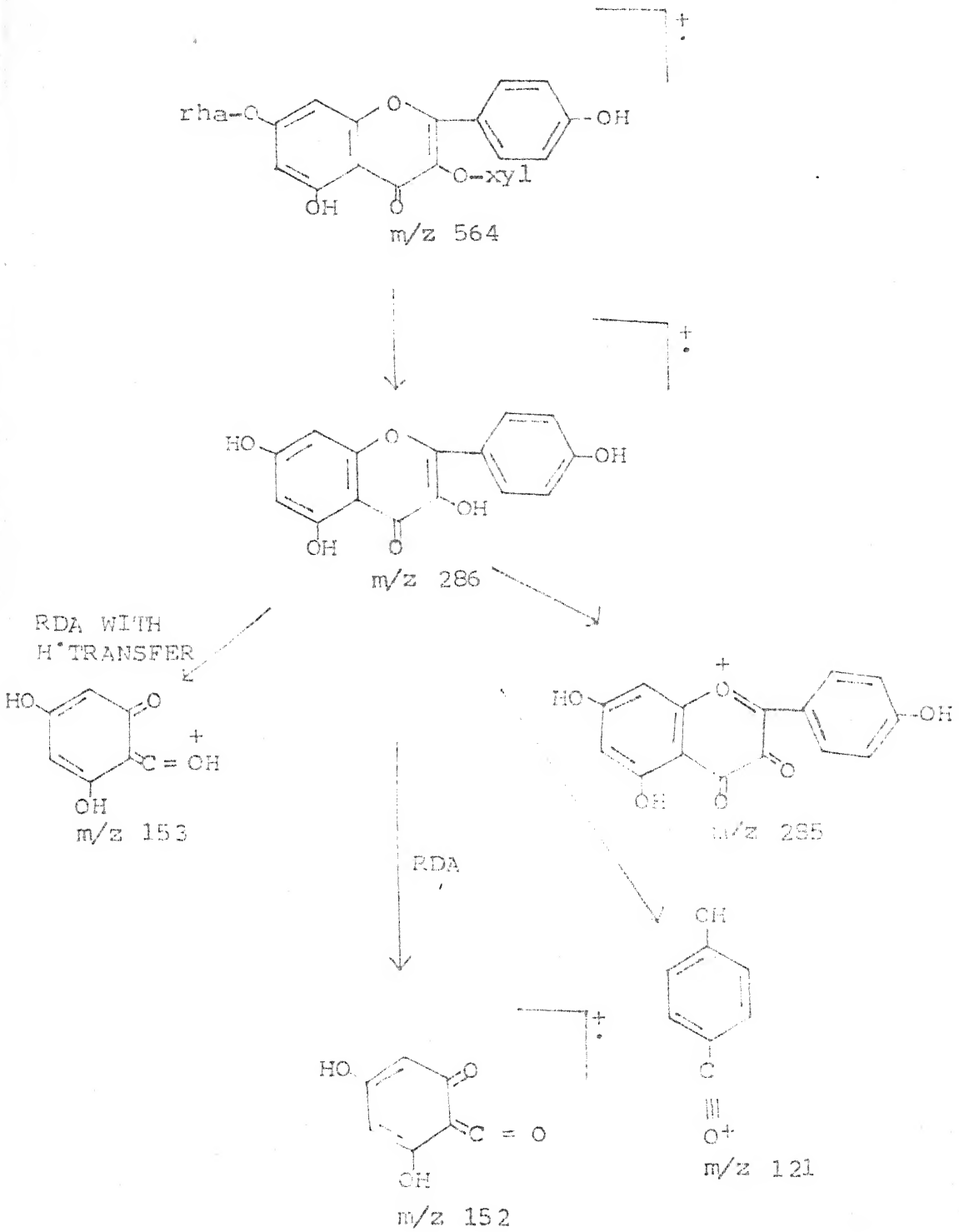
SCHEME - 3 : Mass spectral fragmentation of Quercetin - 7 - O - rhamnoside



SCHEME - 4 : Mass spectral fragmentation of Chrysoeriol - 7 - O - xyloside



SCHEME - 5 : Mass spectral fragmentation of Kaempferol-3-O-xyloside-7-O-rhamnoside



EXPERIMENTAL

All melting points were determined on Kofler Block-Microscope and are uncorrected. The optical rotations were recorded on JASCO DIP-180 automatic polarimeter. The UV spectra were recorded on Hitachi Model-320 automatic recording spectrometer and IR spectra were done on Perkin-Elmer Infra-cord model 157 or on Beckman Acculab-4 infrared spectrophotometer. The NMR spectra were mostly taken on Perkin-Elmer R-32 spectrometer (90 MHz) and on Varian A 60D (60 MHz) in $CDCl_3$ or DMSO-d_6 or Pyridine-d_5 or Acetone-d_6 with TMS as an internal standard. The electron impact and chemical ionisation (CH_4,NH_3) mass spectra were recorded on Jeol D-300 mass spectrometer fitted with JMA-2000 data processing system while field ionisation and field desorption mass spectra were taken on Jeol 01SG-2 mass spectrometer. The gas liquid chromatograms were run on Varian Aerograph, Model instrument 1868-4, using column (3% SE-30 on chromosorb-W), while the HPLC was carried out on Waters Associate Model ALC/GPC-244 liquid chromatograph.

Silica gel G and neutral alumina were used for thin layer chromatography (TLC) and preparative layer chromatography (PLC). PLC were carried out over plates (20x20 cm) coated with 9-10g of silica gel. The spots on TLC and PLC plates were visualised by spraying with 1% potassium permanganate solution, containing few drops of

sulphuric acid or by keeping them for some time in an atmosphere of iodine vapour. For sugars, Whatman paper No.1 was used and spot were visualised with aniline hydrogen phthalate and alkaline potassium permanganate (1% aqueous $KMnO_4$ containing 2% sodium carbonate). For flavones, Whatman No.3mm filter paper were used for paper chromatography and spots were detected by UV light or exposing the paper to ammonia vapours.

ISOLATION OF CONSTITUENTS:

Leaves of Alysicarpus longifolius used in the present investigation were collected from the Institute campus during monsoon season and contained crude protein 20.7%, calcium 2.24% and phosphorus 0.29%. Air dried powdered leaves (1.5 Kg.)were exhaustively extracted with ethanol (95%, 4x3 lit.) by cold percolation. The combined ethanolic extract was concentrated under reduced pressure below 50° and on cooling deposited a yellow crystalline solid which was filtered. The remaining concentrated residue (120 g) was successively macerated with hexane (2x1 lit.), benzene (2x1 lit.) ethyl acetate (2x500 ml), acetone (2x500 ml) and butanol (2x1 lit.) and on concentration afforded the following fractions.

1. Hexane soluble (60 g).
2. Benzene soluble (3.5 g).
3. Ethyl acetate soluble (10 g).
4. Acetone soluble (5 g).
5. n-Butanol soluble (25 g).

COLUMN CHROMATOGRAPHY OF THE HEXANE FRACTION:

The dark green coloured hexane fraction was found to be a complex mixture by TLC. A part of it (40 g) was subjected to column chromatography over silica gel (600 g). The elution was carried out with hexane by increasing proportions of benzene, ethyl acetate followed by ethanol. 110 fractions of 400 ml each were collected and the fractions were monitored by TLC and the results are presented in Table-3.

Table-3 Column chromatography of hexane fraction (40 g)

Fr. No.	Eluant	Weight (g)	Remarks
1-7	Hexane	2.5	Contained substance A
8-10	Hexane:Benzene (90:10)	1.5	Mixture
11-20	Hexane:Benzene (90:10)	1.0	-do-
21-24	Hexane:Benzene (90:10)	0.5	Contained substance B
25-32	Hexane:Benzene (80:20)	3.5	Contained substance C
33-37	Hexane:Benzene (70:30)	2.25	-do-
38-47	Hexane:Benzene (50:50)	1.5	-do-
48-58	Hexane:Benzene (30:70)	1.0	-do-
59-65	Benzene	1.5	Contained substance D

Fr. No.	Eluant	Weight (g)	Remarks
66-68	Benzene	1.0	Contained substance E
69-74	Benzene	1.5	Complex mixture
75-88*	Benzene	1.0	-do-
89-93*	Benzene:Ethyl acetate (90:10)	2.0	-do-
94-95*	Benzene:Ethyl acetate (70:30)	2.5	-do-
96-98*	Benzene:Ethyl acetate (50:50)	2.5	Greenish material
99-101*	Ethyl acetate	2.5	-do-
102-104*	Ethyl acetate:Ethanol (90:10)	2.0	-do-
105-110*	Ethyl acetate:Ethanol (80:20)	1.0	-do-

* Fractions of 1 lit. each.

SUBSTANCE A:

The fractions 1-7 were combined and the solvent removed. The residue (2.5 g) was neutral. It was saponified with 7% ethanolic sodium hydroxide and by usual work up furnished a colourless waxy product of substance A (50 mg) which was recrystallised from benzene. M.p. 65-67°.

IR ν_{max}^{KBr}: 2920, 2850 (-CH-stretching), 1450, 1375 (-CH-bending), 1040, 740 and 720 cm^{-1}.

EIMS: m/z 464, 436, 422, 408, 394, 393, 380, 379, 365, 351, 337, 323, 309, 295, 281, 267, 253, 239, 225, 211, 197, 183, 169, 155, 141, 127, 113, 99, 85, 71, 57 and 43.

FIMS: m/z 464(M^+), 450 (M^+), 436 (M^+), 422 (M^+), 408 (M^+), 394 (M^+) and 380 (M^+).

SUBSTANCE B: Carnaubyl cerotate

The fractions 21-24 were mixed and the solvent removed. The residue (0.57 g) was crystallised from acetone to yield substance B (55 mg). M.p. 72-75°.

IR ν_{max}^{KBr}: 2930, 2850 (-CH-), 1730 (C=O), 1460, 1170, 950, 920, 730 and 720 cm^{-1}.

NMR ($CDCl_3$): δ3.80 (t, 2H, $-OCH_2-$), 2.26 (t, 2H, $-CH_2-CO-$), 1.20 (s, 90H, $-(CH_2)_{45}-$) and 0.85 (deformed t, 3H, $-CH_3$) and 0.65 (deformed t, 3H, $-CH_3$).

EIMS: m/z. 732(M^+), 704, 676,648, 620, 508, 480, 465, 452, 436, 420, 409, 396, 392, 369, 368, 355, 342, 341, 340, 314, 313, 312, 286, 285, 284, 258, 257, 239, 225, 211, 199, 197, 185, 183, 169, 155, 141, 127, 113, 99, 97, 88, 83, 71, 69, 57, 55, 43 and 32.

Hydrolysis of substance B:

Substance B (30 mg) and ethanolic sodium hydroxide (5 ml, 25%) were refluxed for 3 hours. It was cooled and then extracted with ether (3x10 ml) and washed free of alkali. The ether layer was separated, dried (anhydrous Na_2SO_4) and evaporated to afford carnaubyl alcohol (10 mg), crystallised from methanol. M.p. 68°. IR ν_{max}^{KBr}: 3350 (-OH), 2920 (-CH-), 1470, 1060, 740 and 730 cm^{-1}. The aqueous layer on acidification furnished cerotic acid (5 mg), crystallised from ethyl acetate, M.P. 75-76°. IR ν_{max}^{KBr} : 3460 (-OH), 1740 (C=O), 1460, 1060, 730 and 720 cm^{-1}.

SUBSTANCE C: Myricyl alcohol

The fractions 25-58 were combined and the solvent removed. The residue (8.25 g) dissolved in benzene was charcoaled and crystallised from benzene to yield white flakes of substance C (250 mg). M.p. 85-86°.

IR ν_{max}^{KBr} :	3348 (-OH), 2900, 2820(-CH-), 1460, 1050(-CH), 880, 730 and 720 cm^{-1}.
NMR($CDCl_3$):	δ0.89 (t, 3H, $-C\underline{H}_3$), 1.20 (s, 56H, $-(C\underline{H}_2)_{28}-$) and 3.65 (t, 2H, $-C\underline{H}_2OH$).
EIMS:	m/z 420, 406, 392, 378, 364, 350, 335, 321, 307, 293, 279, 265, 251, 237, 223, 209, 195, 181, 167, 153, 139, 125, 111, 97, 83, 69, 57 and 55.
CIMS:	m/z 437 $(M-H)^+$, 421$(M+H-H_2O)^+$.
FDMS:	m/z 438 (M^+).
Analysis:	Found C, 80.6; H, 14.3
$C_{30}H_{62}O$	required C, 81.2; H, 14.1%

Acetylation of Substance C:

A mixture of substance C (50 mg), acetic anhydride (5 ml) and fused sodium acetate (500 mg) was refluxed on an oil bath at 120-30° for 3 hrs, cooled and poured on chilled water. Free acetic acid was neutralised and the aqueous solution was extracted with ether (2x20 ml). The organic layer was washed with water, dried over anhydrous sodium sulphate and the solvent removed. The residue was crystallised from ethanol. M.p. 72°.

IR ν_{max}^{KBr} :	2920, 2840 (-CH-), 1730 ($-OCOCH_3$), 1460, 1360, 1240 and 720 cm^{-1}.

NMR($CDCl_3$): δ 0.89 (t, 3H, $-CH_3$), 1.20 (s, 56H, $-(CH_2)_{28}-$), 2.05 (s, 3H, $-OCOCH_3$) and 4.10 (t, 2H, $-CH_2OAc$).

EIMS: m/z 480 (M^+).

Analysis: Found C, 79.9 ; H, 13.5

$C_{32}H_{64}O_2$ required C, 80.0 ; H, 13.3%.

SUBSTANCE D: β-sitosterol

The fractions 59-65 were concentrated and the solvent evaporated off. The residue (1.5 g) was dissolved in benzene and charcoaled to give substance D (225 mg) which was crystallised from chloroform-methanol.

M.p. 136- 37°, $(\alpha)_D^{25°}$ - 40° (c, 1, $CHCl_3$).

IR ν_{max}^{KBr}: 3400(-OH), 2920(-CH), 1630 (-C=CH-), 1450, 1365, 1050, 950 and 800 cm^{-1}.

NMR($CDCl_3$): δ 5.25(m, 1H, $-C=CH-CH_2-$), 3.40(m, 1H, CHOH), 2.10 (broad m, 1H, -CHOH) and 0.66-1.24(s, 18H, $6xCH_3$).

EIMS: m/z 414 (M^+), 400, 396, 382, 329, 303, 275, 273, 255, 213, 199, 173, 161, 159, 145, 133, 121, 119, 107, 105, 95, 81, 69, 57, 55 and 43.

Analysis: Found C, 84.36 ; H, 12.24

$C_{29}H_{50}O$ required C, 84.06 ; H, 12.07%

Acetylation of substance D:

A solution of β-sitosterol (50 mg) in acetic anhydride (2 ml) and anhydrous pyridine (1 ml) was kept overnight. The solvent was removed under vacuum. The residue was diluted with water. It was extracted with chloroform (3x5 ml). The combined chloroform layer was washed with water, dried over anhydrous Na_2SO_4 and the solvent removed. The residue was crystallised from chloroform as colourless needles (55 mg). M.p. 128-29°, $(\alpha)_D^{25°}$ - 35°($CHCl_3$).

IR ν_{max}^{KBr}:	2900 (CH), 1720($-OCOCH_3$), 1240 and 1040 cm^{-1}.
EIMS:	m/z 456 (M^+).
NMR($CDCl_3$):	δ 5.25 (m, 1H, -C=CH), 4.50 (m, 1H, CHOAc), 1.92 (s, 3H, $-OCOCH_3$), 0.66-1.24 (overlapping s, 18H, 6xCH_3).
Analysis:	Found C, 81.53 ; H, 11.33
$C_{31}H_{52}O_2$	required C, 81.52 ; H, 11.48%.

Substance E: β-sitosterol acetate

The fractions 66-68 were mixed and the solvent evaporated off. The residue (1.5 g) was recrystallised from methanol to afford substance E (10 mg). M.p. 128-129°. The compound was identified as β-sitosterol acetate on the basis of physical data and comparison with authentic sample.

COLUMN CHROMATOGRAPHY OF THE BENZENE FRACTION:

The benzene soluble fraction (3.5 g) was found to be a complex mixture. It was chromatographed over a column of silica gel (50 g). The elution was effected with benzene followed by ethyl acetate, ethanol and their mixtures. The fractions (250 ml each) were mixed on the basis of TLC and the results are recorded in Table 4.

Table-4 Column chromatography of benzene fraction (3.5 g).

Fr. No.	Eluant	Weight (mg)	Remarks
1-3	Benzene	10	Oily residue
4-5	Benzene:Ethyl acetate (90:10)	12	Greenish mixture
6-8	Benzene:Ethyl acetate (90:10)	18	Mixture
9-15	Benzene:Ethyl acetate (70:30)	15	-do-
16-18	Benzene:Ethyl acetate (50:50)	15	-do-
19-21	Benzene:Ethyl acetate (30:70)	70	Contained substance D.
22-24	Ethyl acetate	100	Greenish mixture
25-28	Ethyl acetate:Ethanol (90:10)	300	-do-
29-30	Ethyl acetate:Ethanol (50:50)	150	-do-
31-33	Ethyl acetate: Ethanol (30: 70)	300	-do-
34-36	Ethyl acetate:Ethanol (20:80)	300	Mixture

The fractions 19-21 were mixed on the basis of TLC and the solvent removed. The residue (70 mg) was dissolved in chloroform-methanol (charcoaled) to furnish more of substance D (50 mg).

ETHANOLIC FRACTION:

SUBSTANCE F: Phegopolin

The ethanolic extract on cooling furnished a yellow crystalline solid, which was filtered out. On TLC it showed two spots. The major product was separated by PLC using chloroform-methanol-water (35:9:2) as the developing system to yield substance F (80 mg) as yellow crystals which was recrystallised from methanol. M.p. 203-4°.

IR_{max}^{KBr}:		3400, 1640, 1595, 1555, 1475, 1435, 1340, 1248, 1190, 1165, 1060, 1020, 900 and 830 cm^{-1}.
UV:	(MeOH) λ_{max}	272 and 334 nm.
	(NaOMe)	272 and 334 nm.
	($AlCl_3$)	262, 278, 330 and 380 nm.
	($AlCl_3$/HCl)	262, 278, 330 and 380 nm.
	(NaOAc)	272 and 334 nm.
	(NaOAc/H_3BO_3)	272 and 334 nm.
FDMS:		m/z 469 $(M+Na)^+$ and 447 $(M+H)^+$.
EIMS:		m/z 284 (aglycone), 167, 166, 138, 121 and 118.

Acetylation of substance F:

Substance F (30 mg) was dissolved in dry pyridine and acetic anhydride (1 ml each). The reaction mixture was refluxed for 3 hrs on oil bath at 120°. The solvents were removed under vacuum. The residue was diluted with water (1 ml) and extracted with ether (4x10 ml), dried (anhydrous Na_2SO_4) and the solvent removed. The residue was crystallised from chloroform-methanol to give white silky penta-acetate (35 mg). M.p. 179°.

IR) ν_{max}^{KBr} : 1750, 1650, 1610, 1485, 1440, 1360, 1230, 1160, 1020, 900 and 830 cm^{-1}.

NMR ($CDCl_3$): δ 7.75 (J=10.0 Hz, d, 2H, H-2',6'), 7.13 (J=10.0 Hz, d, 2H, H-3',5'), 7.2 (s, 1H, H-3), 6.5 (J=2.5 Hz, d, 1H, H-8), 6.34 (J=2.54 Hz, d, 1H, H-6), 5.75 (d, H-1''), 4.9-5.35 (m, 6H, H-2'',6''), 3.86 (s, 3H, OCH_3), 2.24 (s, 3H, $-OCOCH_3$), 1.98 (s, 6H, $-OCOCH_3$), 1.92 (s, 3H, $-OCOCH_3$) and 1.69 (s, 3H, $-OCOCH_3$).

EIMS: m/z 656 (M^+).

Hydrolysis of substance F:

20 mg of compound F was refluxed with 10% ethanolic H_2SO_4 (2 ml) for 4 hrs on water bath. The ethanol was

removed and the reaction mixture was extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated. The aglycone was crystallised from methanol as yellow solid. M.p. 280°.

EIMS: m/z 284 $(M)^+$, 167, 166, 138, 121 and 118.

The aqueous portion of hydrolysate was neutralised by barium carbonate. It showed only one spot having the same Rf value as glucose on paper chromatography using butanol-acetic acid-water (4:1:5, v/v, upper) as developing system and aniline hydrogen phthalate as a detecting agent.

ETHYL ACETATE SOLUBLE FRACTION:

The ethyl acetate soluble fraction gave positive Shinoda test (Mg+HCl) indicating the presence of flavonoid compound. It was then concentrated under reduced pressure. The residue (10 g) was dissolved in 100 ml of water and filtered. The filtrate was cooled. A solution of neutral lead acetate was added to it. The yellow precipitate was filtered and washed with water. It was then suspended in ethanol (100 ml) and decomposed by passing H_2S gas (1 hr). The process of decomposition was repeatedthrice and the solvent removed under reduced pressure. The dark brown semi-solid residue was taken up in hot water (50 ml). The aqueous solution decanted off from insoluble dark brown non-flavonoid material and continuously extraced with ethyl acetate (10x250 ml). The combined ethyl acetate

layer was concentrated to a small volume when the mixture of the pigments separated out as brownish yellow solid (60 mg). The neutral lead filtrate was then treated with a solution of basic lead acetate. The yellow lead salt on decomposition and purification as before gave a very small amount of substance G (25 mg).

SUBSTANCE G: Rutin

Crystallised from methanol. M.p. 189-90°.

IR) ν_{max}^{KBr}:		3420, 1655, 1600, 1500, 1360, 1290, 1204, 1090, 1052, 1020 and 800 cm^{-1}.
UV:	(MeOH) λ max	259, 366sh, 299sh and 359 nm.
	(NaOMe)	272, 324 and 410 nm.
	($AlCl_3$)	275, 303sh and 433 nm.
	($AlCl_3$/HCl)	271, 300, 360sh and 402 nm.
	(NaOAc)	272, 325 and 393 nm.
	($NaOAc/H_3BO_3$)	262, 298 and 384 nm.
Analysis		Found C, 48.9 ; H, 5.60
$C_{27}H_{30}O_{16}$		required C, 48.8 ; H, 5.5%.

Hydrolysis of substance G:

A solution of rutin (15 mg) in 3% methanolic sulphuric acid was heated on the water bath for 3 hrs, then methanol was distilled off. The reaction mixture was diluted with water and extracted with ethyl acetate (2x5 ml). The organic layer on concentration furnished a canary yellow

precipitate of aglycone which crystallised from chloroform-methanol (1:2), as yellow granules. M.p. 314-15°.

UV:	(MeOH) λ max	255, 269, 301sh and 370 nm.
	(NaOMe)	247sh and 322 dec nm.
	($AlCl_3$)	272, 304sh, 333 and 455 nm.
	($AlCl_3$/HCl)	259, 301sh, 359 and 429 nm.
	(NaOAc)	257, 274, 329 and 390 dec nm.
	($NaOAc/H_3BO_3$)	261, 303sh and 388 nm.
NMR (Acetone-d_6):		δ 7.71 (J=2.5 Hz, d, 1H, H-2'), 7.59 (J=9 Hz and 2.5 Hz, dd, 1H, H-6'), 6.86 (J=9 Hz, d, 1H, H-5'), 6.38 (J=2.5 Hz, d, 1H, H-8) and 6.16 (J=2.5 Hz, d, 1H, H-6).
EIMS:		m/z 302(M^+), 301, 153 and 137.

Identification of the sugar residue:

The hydrolysate after separation of aglycone was neutralised with barium carbonate. The solution was concentrated. The sugar moiety as glucose and rhamnose was confirmed by paper chromatography, using n-butanol-acetic acid-water (4:1:5, v/v, upper) as a developing system and aniline hydrogen phthalate as a spraying reagent.

Separation of the flavonoid glycosides by paper chromatography:

The mixture of pigments (60 mg) obtained from the neutral lead - acetate fraction was placed on Whatman No.3 mm filter paper and ascending chromatography performed at room temperature using n-butanol-acetic-acid water (4:1:5, v/v, upper) as a developing solvent. The chromatograms were run for eighteen hours. They were air dried and zones were detected with UV light. The upper and lower zones corresponding to the pigments H and I. These were then separately cut and extracted with methanol (2x50 ml, 6 hrs, each time). The combined methanol extracts from each zone were concentrated to 4-5 ml, when the pigments separated out as substance H (20 mg, Rf=0.76) and I (15 mg Rf=0.52).

SUBSTANCE H: Quercetin-7-O-rhamnoside

Crystallised from methanol as yellow granules. M.p.223-25°.

IR ν_{max}^{KBr}:		3400, 1650, 1610, 1580, 1500, 1430, 1350, 1230, 1024, 975, 890 and 770 cm^{-1}.
UV:	(MeOH) λ max	256, 272 and 347 nm.
	(NaOMe)	278, 337sh and 410 nm.
	($AlCl_3$)	275, 302sh, 332 and 420 nm.
	($AlCl_3$/HCl)	262sh, 276, 302sh, 356 and 400 nm.
	(NaOAc)	272, 284, 360 and 428 nm.
	(NaOAc/H_3BO_3)	262, 289sh and 372 nm.
FDMS:		m/z 471 $(M+Na)^+$, 449 $(M+H)^+$ and 303 $(aglycone+H)^+$.
EIMS:		m/z 302, 301, 153, 152 and 137.

Acid hydrolysis of substance H:

Substance H (10 mg) was refluxed in 6% methanolic HCl for 4 hrs. The reaction mixture was extracted with ethyl-acetate (3x2 ml). The organic layer was washed with water, dried over sodium sulphate and concentrated. The aglycone so obtained from the organic layer was identified as quercetin by comparison with an authentic sample. The aqueous layer was neutralised with barium carbonate and the sugar was confirmed as rhamnose by paper chromatography in n-butanol-acetic acid-water (4:1:5, v/v, upper) as developing solvent and aniline hydrogen phthalate as a spraying agent.

FDMS: Aglycone m/z 302 (M^+).

Sugar m/z 165 $(M+H)^+$.

SUBSTANCE I: Chrysoeriol-7-O-xyloside

Obtained as a yellow microcrystalline product.

IR ν_{max}^{KBr}: 3400, 1650, 1615, 1580, 1440, 1375, 1350, 1280, 1185, 1080, 840 and 775 cm^{-1}.

UV: (MeOH) λ_{max} 272, 310 and 332 nm.

(NaOMe) 276, 330 and 372 nm.

($AlCl_3$) 260, 276, 302, 350 and 384 nm.

($AlCl_3$/HCl) 260, 280, 302, 342 and 380 nm.

(NaOAc) 272 and 332 nm.

(NaOAc/H_3BO_3) 271 and 332 nm.

FDMS: m/z 455 $(M+Na)^+$, 433 $(M+H)^+$ and 301 (aglycone +H)$^+$.

EIMS: m/z 300, 153, 152, 151 and 148.

Acid hydrolysis of substance I:

Substance I (10 mg) was refluxed with methanolic 6% HCl for 4 hrs. The reaction mixture was extracted with ethyl acetate (2x3 ml). The ethyl acetate layer was washed with water, dried over sodium sulphate and concentrated. The aglycone obtained from organic layer was crystallised from methanol. M.p. 328°. The aqueous layer after neutralisation with barium carbonate showed the presence of xylose by paper chromatography using n-butanol-acetic acid-water (4:1:5, v/v, upper) as a developing system and aniline hydrogen phthalate as a spraying agent.

UV:	(MeOH)	λ max	241, 249sh, 269, 330 and 347 nm.
	(NaOMe)		262, 275, 325sh and 392 nm.
	($AlCl_3$)		262, 274, 300, 342 and 390 nm.
	($AlCl_3$/HCl)		259, 276, 296, 340 and 386 nm.
	(NaOAc)		271, 320 and 392 nm.
	(NaOAc/H_3BO_3)		268 and 349 nm.
FDMS:	Aglycone		m/z 301 $(M+H)^+$.
	Sugar		m/z 151 $(M+H)^+$.

COLUMN CHROMATOGRAPHY OF BUTANOL FRACTION:

The brown viscous butanol soluble fraction on TLC showed complex mixture. A part of it (20 g) was chromatographed over silica gel (350 g). The elution was effected by ethyl acetate in increasing proportions of methanol, butanol and butanol saturated with water. The fractions (500 ml each) were combined on the basis of TLC and the results are summarised in Table-5.

Table-5 Column chromatography of the butanol fraction (20 g).

Fr. No.	Eluant	Weight (g)	Remarks
1-3	Ethyl acetate	0.40	Mixture
4-7	Ethyl acetate:Methanol (90:10)	0.50	-do-
8-14	Ethyl acetate:Methanol (90:10)	0.60	Contained substance J
15-16	Ethyl acetate:Methanol (80:20)	0.60	-do-
17-22	Ethyl acetate:Methanol (70:30)	0.80	Contained substance K
23-27	Ethyl acetate:Methanol (60:40)	1.0	Complex Mixture
28-29	Ethyl acetate:Methanol (50:50)	1.5	-do-
30-31	Ethyl acetate:Methanol (40:60)	1.5	Syrupy residue
32-33	Ethyl acetate:Methanol (30:70)	1.0	-do-
34-35	Ethyl acetate:Methanol (20:80)	1.0	-do-
36-38	Methanol	1.5	-do-
39-40	Methanol:Butanol (80:20)	2.0	Contained free sugars
41-42	Methanol:Butanol	2.0	-do-
43-44	Butanol saturated with water	2.0	-do-

SUBSTANCE J: D (+) Pinitol

Fractions 8-16 were combined and concentrated to 5 ml. Addition of acetone (3 ml) and on keeping in refrigerator a crystalline solid deposited which crystallised from acetone-methanol mixture as colourless crystals (120 mg). M.p. 186-87°, $(\alpha)_D^{22°}$ +67° (H_2O).

IR ν_{max}^{KBr} :	3380, 3330, 2920, 2880, 1470, 1440, 1370, 1330, 1270, 1240, 1120, 1080, 1040, 1010, 990, 950, 895, 855, 750 and 700 cm^{-1}.
FDMS:	m/z 217 $(M+Na)^+$ and 195 $(M+H)^+$.
EIMS:	176, 158, 144, 126, 116, 105, 103, 97, 88, 87, 85, 74, 73, 69, 60, 57, 45, 43, 33 and 29.
Analysis:	Found C, 43.70 ; H, 7.48
$C_7H_{14}O_6$	required C, 43.35 ; H, 7.21%.

Acetylation of substance J:

A solution of compound J (50 mg), dry pyridine and acetic anhydride (1.5 ml each) was refluxed on an oil bath for 4 hrs at 120°. After removal of the solvents under vacuum the residue was diluted with cold water (1.5 ml) and extracted with ether (4x10 ml). The combined ether extract was dried over anhydrous sodium sulphate and on concentration afforded a solid which was recrystallised from methanol to

furnish the acetate (35 mg). M.p. 98°.

IR ν_{max}^{KBr}: 1730, 1370, 1210, 1060, 920 and 740 cm^{-1}.

SUBSTANCE K: Kaempferol-3-O-xyloside-7-O-rhamnoside

The fractions 17-32 were mixed and concentrated to 15 ml. Addition of ethanol (2 ml) and keeping it in refrigerator for three days yielded yellow crystalline solid (70 mg), which was filtered out. It responded to positive Shinoda test (Mg+HCl) suggesting the presence of flavonoid compound. It showed three spots on paper chromatography using butanol-acetic-acid-water(4:1:5,v/v,upper) as a developing system and ammonia and UV light as a detecting agents. Separation of the substance K (25 mg) was done in the same way by paper chromatography as discussed previously in the ethyl acetate fraction. It was crystallised from methanol. M.p. 237°(dec).

IR ν_{max}^{KBr}: 3380, 1650, 1580, 1556, 1380, 1240, 1180, 1060, 1020 and 830 cm^{-1}.

UV:

(MeOH)	λmax	272, 302sh and 334 nm.
(NaOMe)		282, 332sh and 400 nm.
($AlCl_3$)		280, 304, 350 and 380 nm.
($AlCl_3$/HCl)		280, 302, 345 and 379 nm.
(NaOAc)		280, 300sh and 380 nm.
(NaOAc/H_3BO_3)		272, 302sh and 330 nm.

NMR (DMSO-d_6): δ7.80 (J=9 Hz, d, 2H, H-2',6'), 6.8 (J=9 Hz, d, 2H, H, -3',5'), 6.72 (J=2.5 Hz, d, 1H, H-8), 6.28 (J=2.5 Hz, d, 1H, H-6) and rhamnose $-CH_3$ at 1.20 (m, 3H, CH_3).

FDMS: m/z 587 $(M+Na)^+$, 565 $(M+H)^+$, 455 $(M+Na-132)^+$, 433 $(M+H-132)^+$ and 419 $(M+H-146)^+$.

EIMS: m/z 286 (aglycone), 285, 153, 152 and 121.

Acid hydrolysis of substance K:

Substance K (15 mg) was refluxed with methanolic 6% HCl for 4 hrs. The reaction mixture was extracted with ethyl acetate (3x3 ml), washed with water, dried over sodium sulphate and concentrated. The aglycone obtained from the organic layer was crystallised from methanol. M.p. 279°. The aqueous layer after neutralisation with barium carbonate showed the presence of xylose and rhamnose by paper chromatography using n-butanol-acetic acid-water (4:1:5, v/v, upper) as a developing system and aniline hydrogen phthalate as a spraying reagent.

UV:	(MeOH)	λmax	253, 272, 298sh and 327 nm.
	(NaOMe)		278, 290sh, 326 and 394 nm.
	($AlCl_3$)		278, 304sh, 342 and 390 nm.
	($AlCl_3$/HCl)		280, 304sh, 330 and 390 nm.
	(NaOAc)		280, 304 and 370 nm.
	(NaOAc/H_3BO_3)		284, 320sh and 352 nm.
FDMS:	Aglycone		m/z 286 (M^+).
	sugar		m/z 165 $(M+H)^+$ and 151 $(M+H)^+$.

IDENTIFICATION OF FREE SUGARS:

The fractions 30-44 were combined and concentrated under vacuum to give a brown syrupy product. It was applied as a spot on Whatman No.1 filter paper alongwith authentic sugar samples. The chromatograms were developed in two different solvent system i.e. n-butanol-acetic acid-water (4:1:5, v/v, upper) and n-butanol-ethanol-water (4:1:5, v/v, upper) and run for 16 hrs by the descending technique. After drying the chromatograms were sprayed with aniline hydrogen phthalate and heated in an oven at 105°, when bright brown spots were observed. This fraction showed the presence of two spots having identical Rf values with that of D-glucose and xylose. Identity of the sugar as D-glucose and xylose was further confirmed by co-chromatography with the authentic samples of D-glucose and xylose.

INVESTIGATION OF ALYSICARPUS LONGIFOLIUS SEEDS

INTRODUCTION:

A. longifolius an annual herb is used as a fodder in Bundelkhand. The seeds of the plant are quite nutritious and fed to livestock as concentrate alongwith other roughages. No work has been reported on its seeds. Therefore, a study was undertaken on the composition of its fatty oil and amino acid composition of the seed meal.

RESULTS AND DISCUSSION:

Seeds were found to contain fairly good amount of crude protein (31.8%) and a yellow coloured fixed oil (8.39%) with a characteristic aroma. Examination of the oil by gas liquid chromatography revealed the presence of seven fatty acids. It has a high percentage of palmitic acid (49.1%) and displays a high degree of saturation (66.4%). The UV spectrum showed low level of conjugated dieonic acids (2.36%) while all other conjugated isomers were present in insignificant amount.

Analysis of the defatted seed powder showed the presence of essential and non-essential amino acids with high percentage of glutamic acid, aspartic acid, arginine, and leucine, while the percentages of the other amino acids were relatively low. Methionine and cystine were present in very low concentration. However, other essential amino acids were present in moderate quantities.

EXPERIMENTAL

Mature seeds of A.longifolius were collected from the Institute campus, dried in a vacuum oven at 35°. The seeds were ground to 20 mesh after removal of the seed coat. The crude protein content (31.8%) of powdered seeds was determined by Kjeldahl method[84]. It was defatted with petroleum ether (b.p. 40-60°) in an soxhlet extractor and on concentration the extract afforded a yellow viscous fixed oil (8.39%). It was decolourised by charcoal and fuller's earth. A part of the oil was subjected to gas liquid chromatography under following conditions.

1. Column - 10% silar 7 C P on Chrom. W 6 ft.x1/8 in o.d.
2. Column temperature - 210°.
3. Injector temperature - 240°.
4. Carrier gas - Nitrogen.
5. Flow rate - 50 ml/min.
6. Flame ionization detector.

The oil contained the following percentage of fatty acids:-

1. Laurate (C 12:0)	4.7%
2. Myristate (C 14:0)	3.3%
3. Palmitate (C 16:0)	49.1%
4. Stearate (C 18:0)	9.3%
5. Oleate (C 18:1)	12.3%
6. Linoleate (C 18:2)	17.7%
7. Linolenate (C 18:3)	3.6%

Further in order to determine the percentage of conjugated fatty acids, the method described in AOAC 1960[84] was adopted. The oil (0.334 g) dissolved in one litre of cyclohexane was studied by UV at specified wavelength. The results presented as follows.

1. 233 nm - .813 ABS
2. 262 nm - .360 ABS
3. 268 nm - .341 ABS
4. 274 nm - .360 ABS
5. 308 nm - .158 ABS
6. 315 nm - .105 ABS
7. 322 nm - .114 ABS
8. 346 nm - .048 ABS

The percentage of dieonic acids was 2.36% and other conjugated fatty acids (i.e. trienoic, tetraenoic and pentaenoic) were present in insignificant amount.

The defatted seed powder (500 mg) was hydrolysed with 6N HCl (2 ml) in sealed tube for 24 hrs and than subjected to analysis on a Durrum D 500 amino acid analyser. Tryptophan was not determined. The results are reported in Table-1 in mg amino acid/ g N and percentage of nitrogen was found to be 8.85%.

Table-1

Amino acids	mg amino acid/ g N
Isoleucine	205
Leucine	381
Lysine	263
Methionine	57
Cystine	84
Phenylalanine	235
Tyrosine	180
Threonine	171
Valine	226
Arginine	563
Histidine	147
Alanine	197
Aspartic acid	626
Glutamic acid	939
Glycine	206
Proline	227
Serine	252

CHAPTER-II

THE CHEMICAL INVESTIGATION OF *ALYSICARPUS BUPLEURIFOLIUS* DC.

Alysicarpus bupleurifolius

hexane, benzene, ethyl acetate and acetone soluble fractions. Each fraction was subjected to detailed chemical investigation. The column chromatography of the fractions over silica gel followed by preparative layer chromatography resulted in the isolation of following constituents as summarised in Table-1.

Table-1

Sl. No.	Name of Constituents	Molecular formula	m.p.
1.	Substance A		65-67°
	a) Heptacosane	$C_{27}H_{56}$	
	b) Octacosane	$C_{28}H_{58}$	
	c) Nonacosane	$C_{29}H_{60}$	
	d) Triacontane	$C_{30}H_{62}$	
	e) Hentriacontane	$C_{31}H_{64}$	
	f) Dotriacontane	$C_{32}H_{66}$	
	g) Tritriacontane	$C_{33}H_{68}$	
	h) Pentatriacontane	$C_{35}H_{72}$	
2.	Substance B		78-84°
	a) Tetratricontanol	$C_{34}H_{70}O$	
	b) Tritriacontanol	$C_{33}H_{68}O$	
	c) Dotriacontanol	$C_{32}H_{66}O$	
	d) Triacontanol	$C_{30}H_{62}O$	
	e) Nonacosanol	$C_{29}H_{60}O$	
	f) Octacosanol	$C_{28}H_{58}O$	
	g) Tetratriacontane	$C_{34}H_{70}$	

Sl. No.	Name of Constituents	Molecular formula	m.p.
	h) Dotriacontane	$C_{32}H_{66}$	
	i) Hentriacontane	$C_{31}H_{64}$	
	j) Triacontane	$C_{30}H_{62}$	
	k) Octacosane	$C_{28}H_{58}$	
3.	Substance C β - sitosterol-β-D -glucoside	$C_{35}H_{60}O_6$	300°
4.	Substance D Pinitol	$C_7H_{14}O_6$	186-87°
5.	Substance E Meso-inositol	$C_6H_{12}O_6$	218-19°
6.	Free Sugars		
	a) Glucose		
	b) Arabinose		
	c) Sucrose		
7.	Free amino acids		
	I. Essential amino acids		
	a) Leucine		
	b) Isoleucine		
	c) Threonine		
	d) Methionine		
	e) Tryptophan		
	II. Non essential aminoacids		
	a) Glutamic acid		
	b) Alanine		
	c) Serine		
	d) Aspartic acid		
	e) Glycine		
	f) Histidine		

RESULTS AND DISCUSSION

Substance A: m.p. 66-67°. Crystallised from benzene as micro-crystalline solid. Its aliphatic nature was evident from spectroscopic studies. The IR spectrum showed no functionality suggesting the possibility of it being a straight chain saturated hydrocarbon[52]. However, GLC analysis (Fig.1) showed it to be a mixture of mainly seven components. Substance A was identified as a mixture of the following hydrocarbons[53-57,86] i.e. heptacosane (1), octacosane (2), nonacosane (3), triacontane (4), hentriacontane (5), dotriacontane (6), tritriacontane (7) and pentatriacontane (8) by GLC, electron impact and field ionisation mass spectral studies (Fig.2 and 3) as discussed in Chapter I. The approximate percentage composition of the mixture as found from GLC and FIMS data is presented in Table-2.

Table-2

Peak No.	GLC DATA %	FIMS DATA %	Molecular Wt.
1	1.2	5.4	380
2	1.1	2.3	394
3	4.2	5.1	408
4	2.2	2.5	422
5	71.6	54.4	436
6	2.3	5.4	450
7	17.2	20.4	464
8	-	4.2	492

FIG-1 GLC OF SUBSTANCE-A

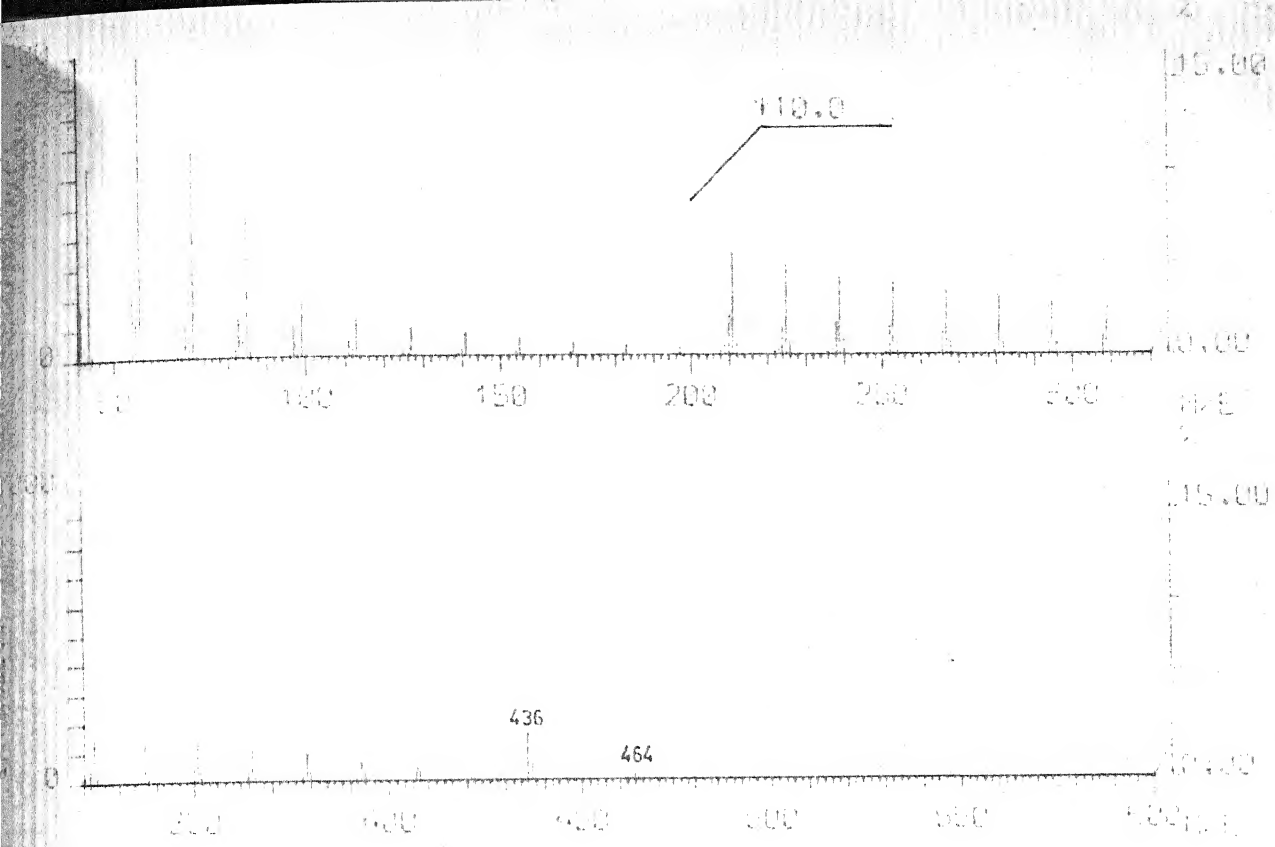


FIG-2 EI MASS SPECTRUM OF SUBSTANCE-A.

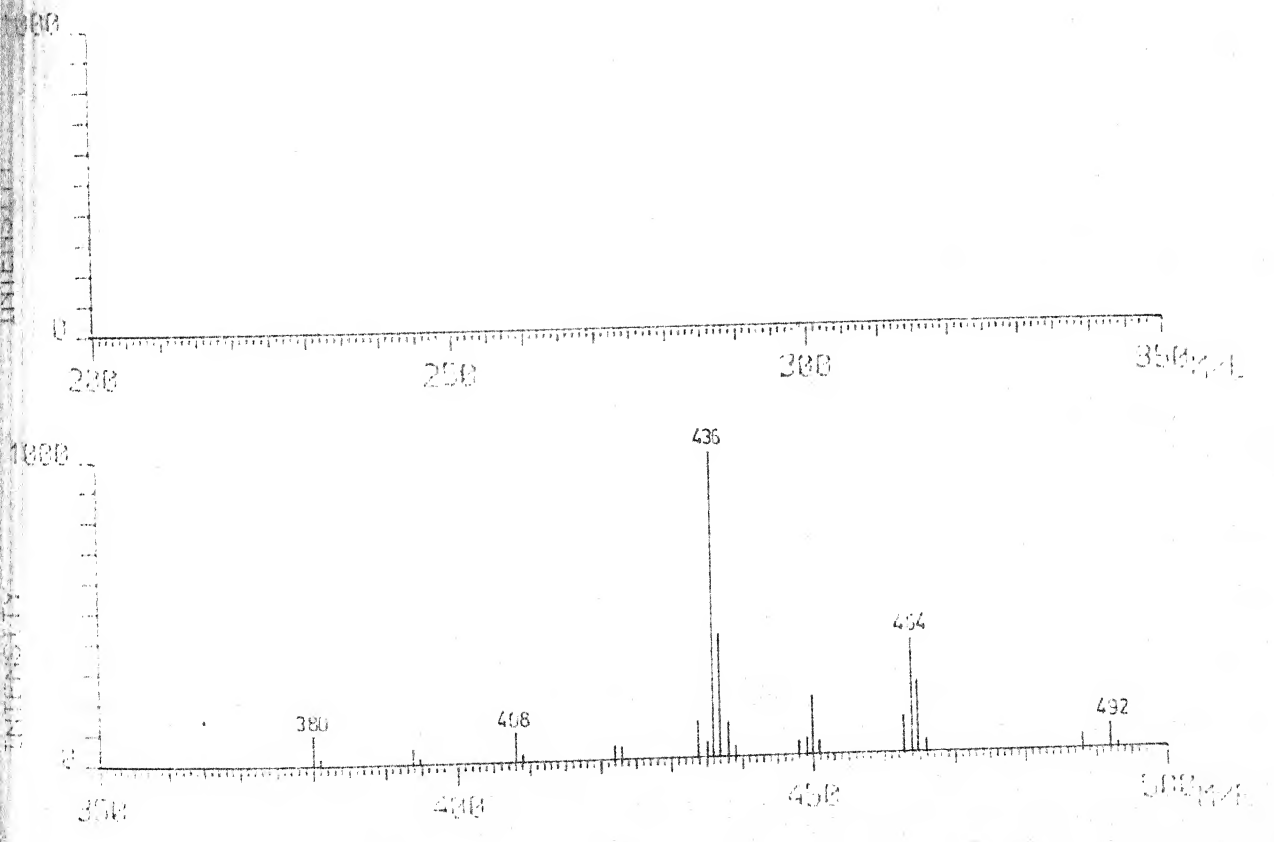


FIG-3 FI MASS SPECTRUM OF SUBSTANCE-A.

Substance B: m.p. 83-84°. Crystallised from hexane as colourless waxy solid. The bands at 3400 and 1050 cm^{-1} in the IR spectrum (Fig.4) indicated the presence of hydroxy group. The bands at 2900, 2850, 1450, 1375 cm^{-1} further showed the presence of -CH- stretching and bending vibrations. The electron impact mass spectrum showed a series of peaks at odd mass corresponding to $C_nH^+_{2n+1}$, $C_nH^+_{2n-1}$ and $C_nH_{2n+1}O^+$ ions and prominent even mass ion peaks at m/z 392, 406, 420, 448 and 476 (Fig.5). These peaks correspond to the molecular ions of the olefins produced by dehydration of the corresponding alcohols. Hence substance B seems to be a mixture of straight chain aliphatic alcohols. The field desorption mass spectrum of the substance B was obtained to confirm the molecular weights and thus the identity of the components. Peaks at m/z 394, 410, 422, 424, 436, 438, 450, 466, 478, 480 and 494 were observed (Fig.6). The peaks at m/z 394, 422, 436, 450 and 478 showed the presence of saturated hydrocarbons, while the peaks at m/z 410, 424, 438, 466, 480 and 494 established the identity of alcohols. The substance B formed an acetate. The IR spectrum of acetate displayed bands at 1730 and 1260 cm^{-1} for the acetoxy function. The EI mass spectrum of the acetate besides giving the aliphatic series of ions also showed peaks at 392, 406, 420, 448, 476, 480, 508 and 536. The chemical ionisation mass spectra (CH_4) of the acetate showed the following series of peaks in the molecular weight region (Fig.7).

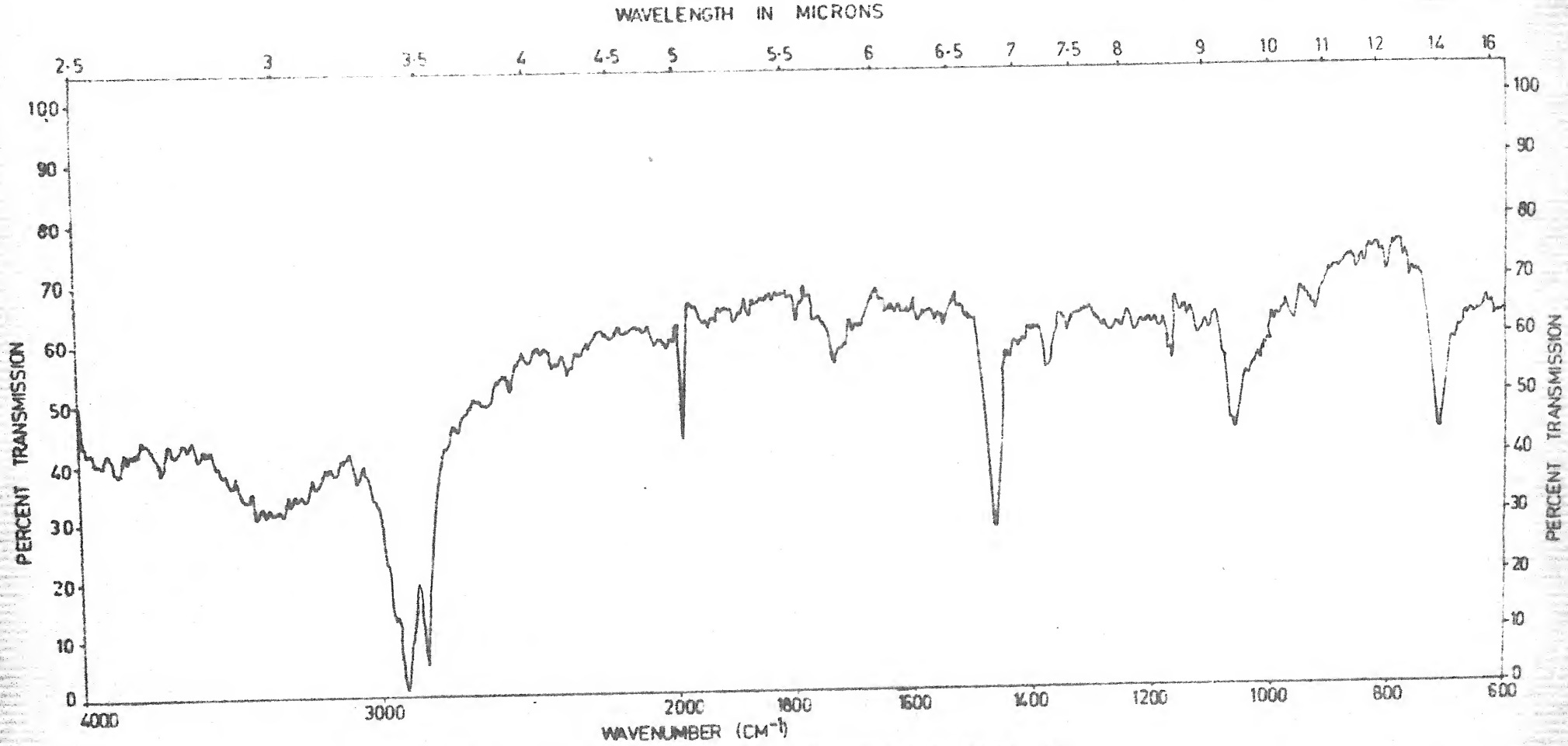


FIG-4 IR SPECTRUM OF SUBSTANCE-B.

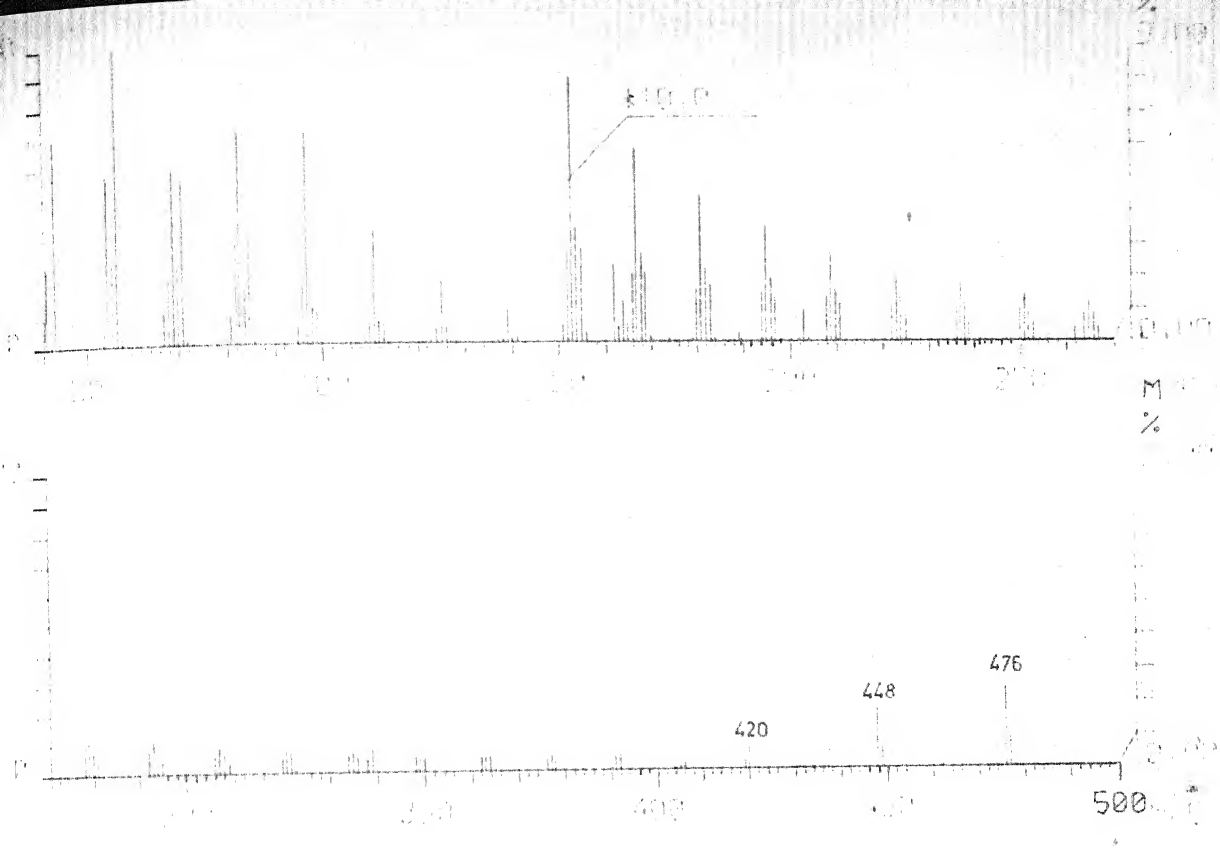


FIG-5 EI MASS SPECTRUM OF SUBSTANCE-B.

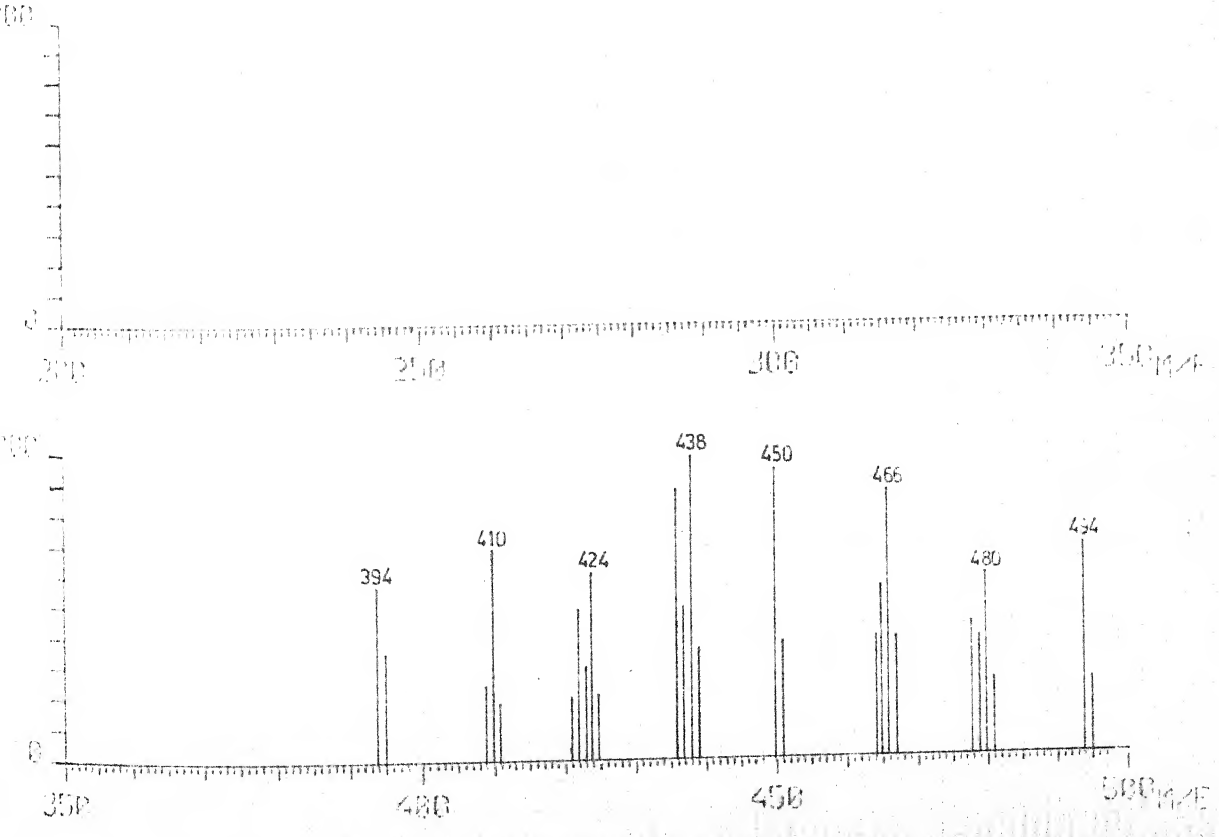


FIG-6 FD MASS SPECTRUM OF SUBSTANCE-B.

$(M+H)^+$: m/z 453, 467, 481, 509, 523 and 537.

$(M-H)^+$: m/z 451, 465, 479, 507, 521 and 535.

$(M+H-60)^+$: m/z 393, 407, 421, 449, 463 and 477.

The CIMS data confirmed the molecular weights of the alcohols. The peaks at m/z 394, 422, 436, 450, 478 due to hydrocarbon were still present in the FDMS of the acetate (Fig.8). The peaks at m/z 452, 466, 480, 507, 522, 536 represent the molecular ions of the acetates of the respective alcohols. Thus the compound B was found to be a mixture of following hydrocarbons and alcohols[87-92].

Hydrocarbons: Octacosane, triacontane, hentriacontane, dotriacontane and tetratricontane[93] (9).

Alcohols : Octacosanol (10), nonacosanol (11), triacontanol (12), dotriacontanol (13), tritriacontanol (14) and tetratriacontanol (15).

Substance C: It was first isolated as a solid mass which on repeated crystallisation from methanol gave colourless crystals m.p. 300°, $(\alpha)_D^{25°}$ -43° (pyridine). It is sparingly soluble in methanol, ethanol and insoluble in hexane, petroleum ether and benzene. A sharp peak at 3400 cm^{-1} and another broad peak at 1020 cm^{-1} in the IR spectrum indicated the presence of hydroxyl group and glycosidic linkage respectively in the molecule. It gave positive Liebermann-Burchard test[61] (purple-green) for sterols and also responded positively to Fiegel

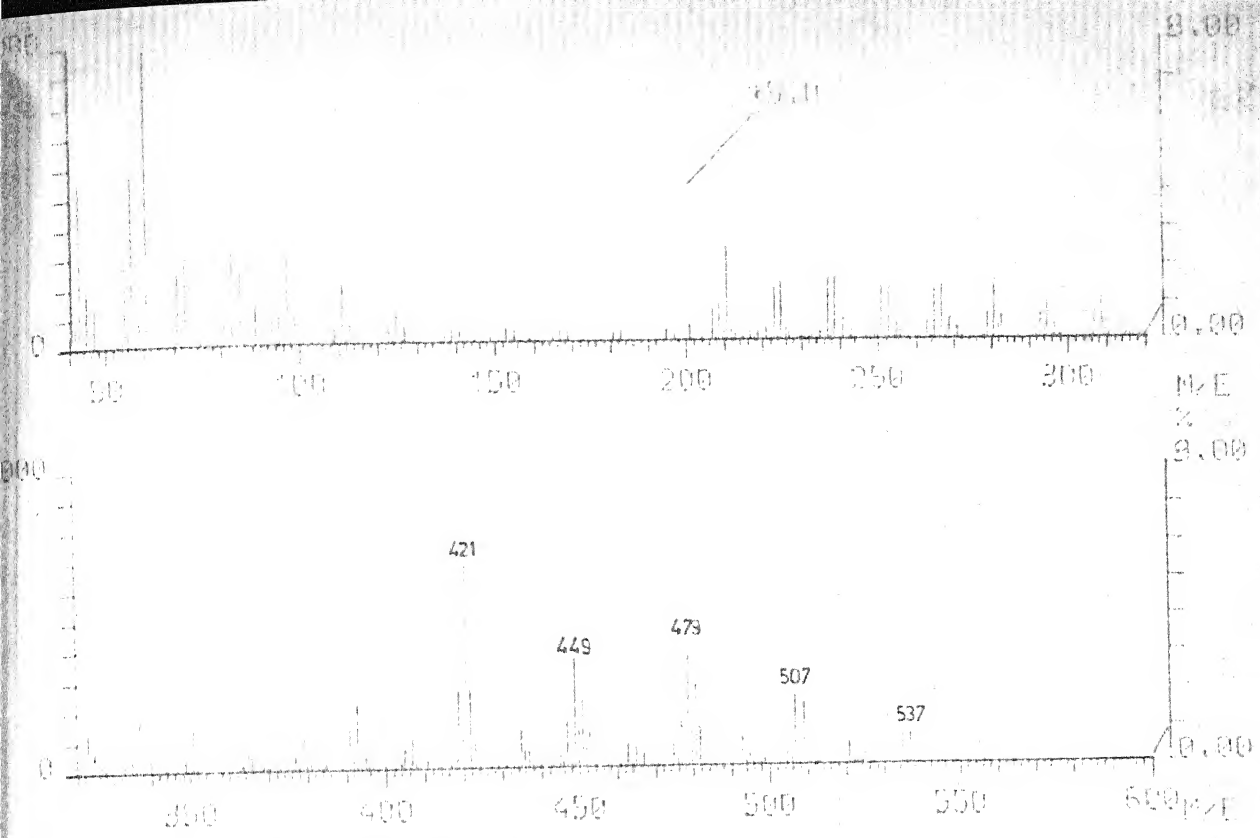


FIG-7 CI MASS SPECTRUM
ACETATE OF SUBSTANCE-B.

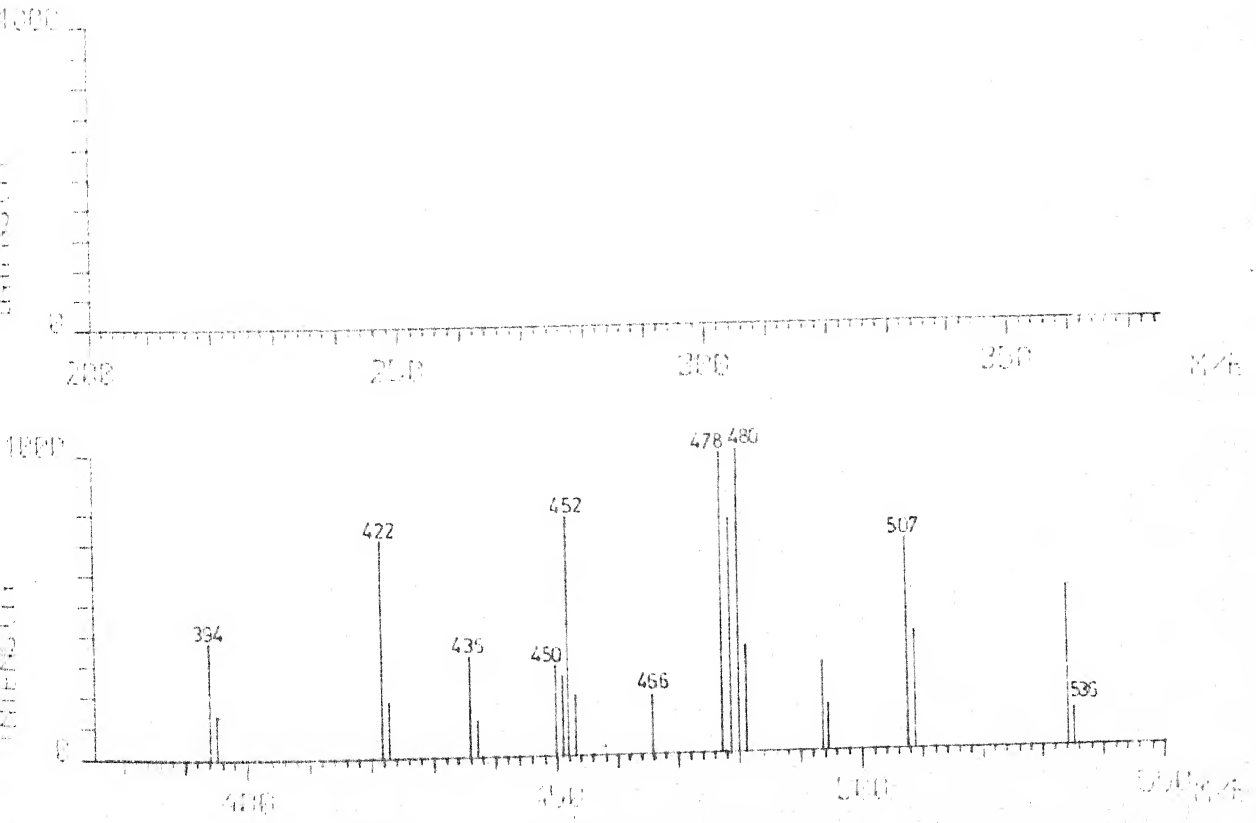


FIG-8 FD MASS SPECTRUM
ACETATE OF SUBSTANCE-B.

colour test[65]. These two tests when considered together led to the conclusion that this compound is a sterioidal glycoside. On acetylation with acetic anhydride and pyridine, it gave a tetra acetate, m.p. 168-179° which was analysed for $C_{43}H_{68}O_{10}$. The electron impact mass spectrum (Fig.9) of the acetate gave a peak corresponding to the molecular ion at m/z 744 (M^+) and other peaks at m/z 684, 624, 564 and 504 due to successive losses of acetic acid molecules thus showing that the substance has formed a tetra-acetate. Hence the molecular weight of substance C was confirmed to be 576. The presence of glucose is indicated by intense peaks at m/z 169 and 331 which are typical of acetylated glucose[41]. Hence, it appears that the substance C is a glucoside. The ammonia chemical ionisation mass spectrum (Fig.10) of the acetate showed three intense peaks at m/z 762 $(M+NH_4)^+$, 397 $(M+NH_4-365)^+$, 366 (glucose tetra-acetate+NH_4)$^+$. These observations further confirmed the substance C as β-sitosterol glucoside. Its NMR spectrum (Fig.11) showed four singlets at δ 1.94, 1.95, 1.97 and 1.99, indicating the presence of four acetoxy groups, two multiplets at δ 4.1 and 4.5 were due to glucosyl protons attached to (C-1' and C-2'). Further, a broad multiplet stretched between δ 4.7-5.1 was attributed to the glucosyl protons attached to (C-3', C-4', C-5' and C-6').

Hydrolysis of the glycoside with 5% ethanolic H_2SO_4 gave the aglycone m.p. 136°, identified as

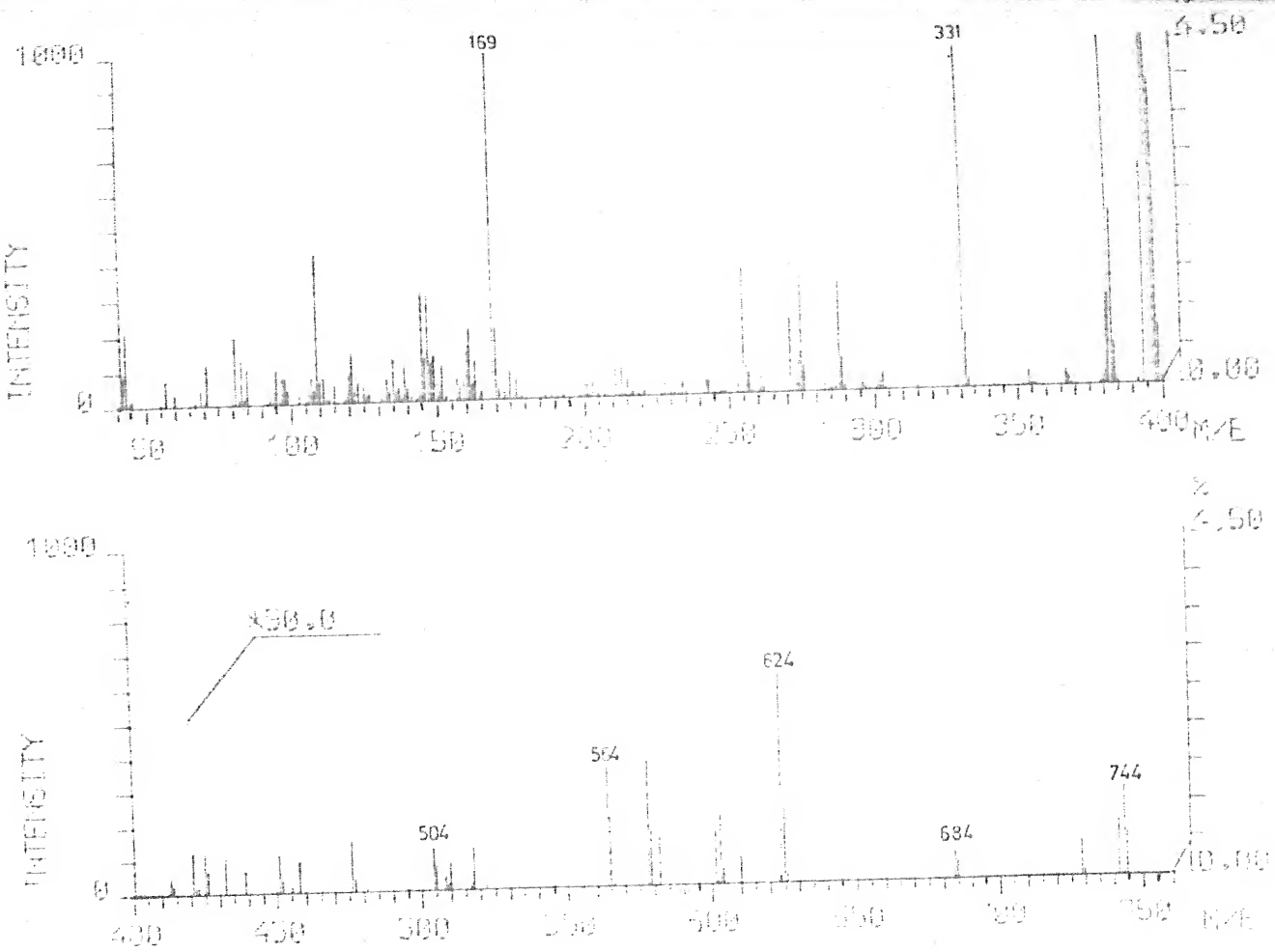


FIG-9 EI MASS SPECTRUM
ACETATE OF SUBSTANCE-C

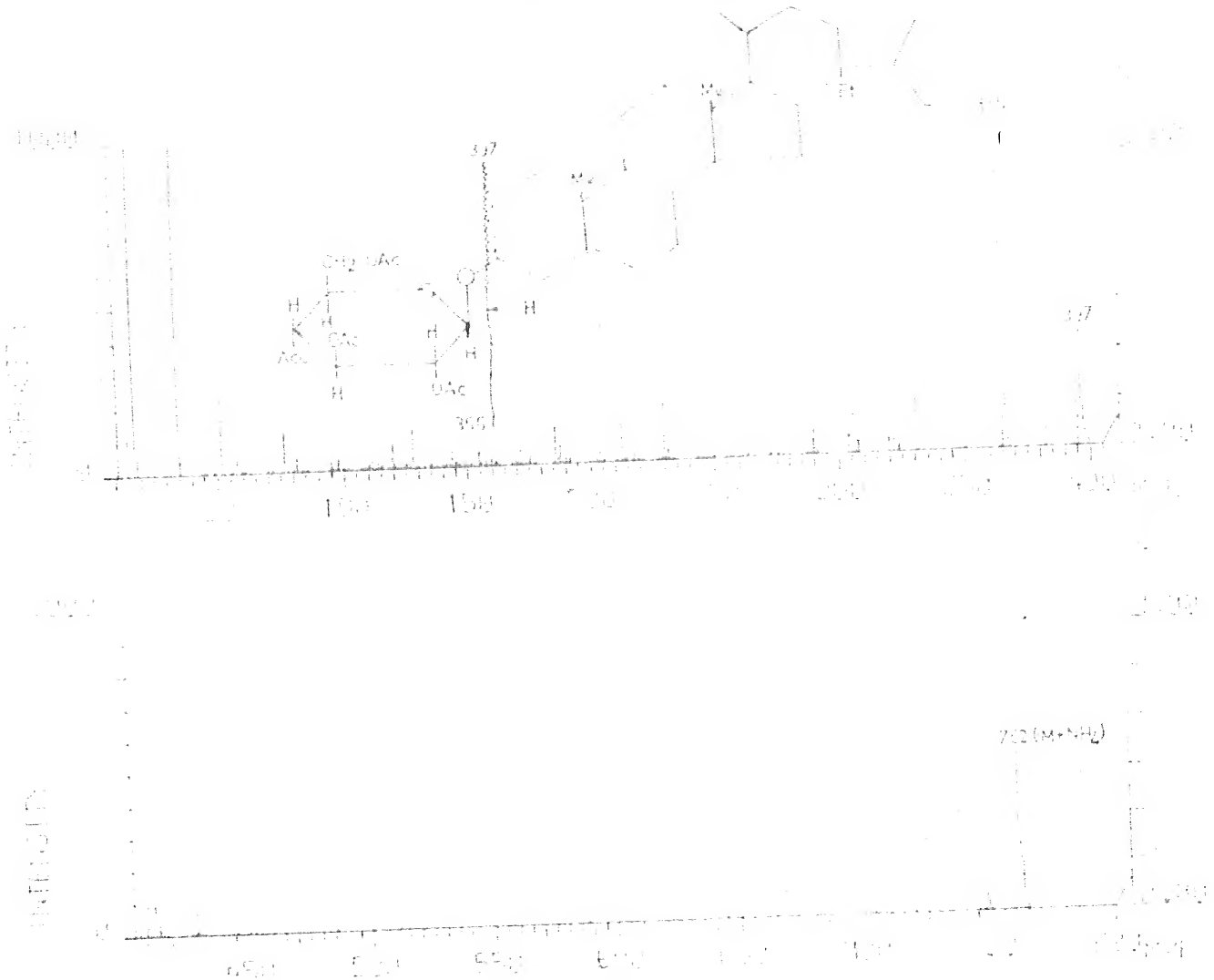


FIG-10 C I AMMONIA MASS SPECTRUM
ACETATE OF SUBSTANCE-C

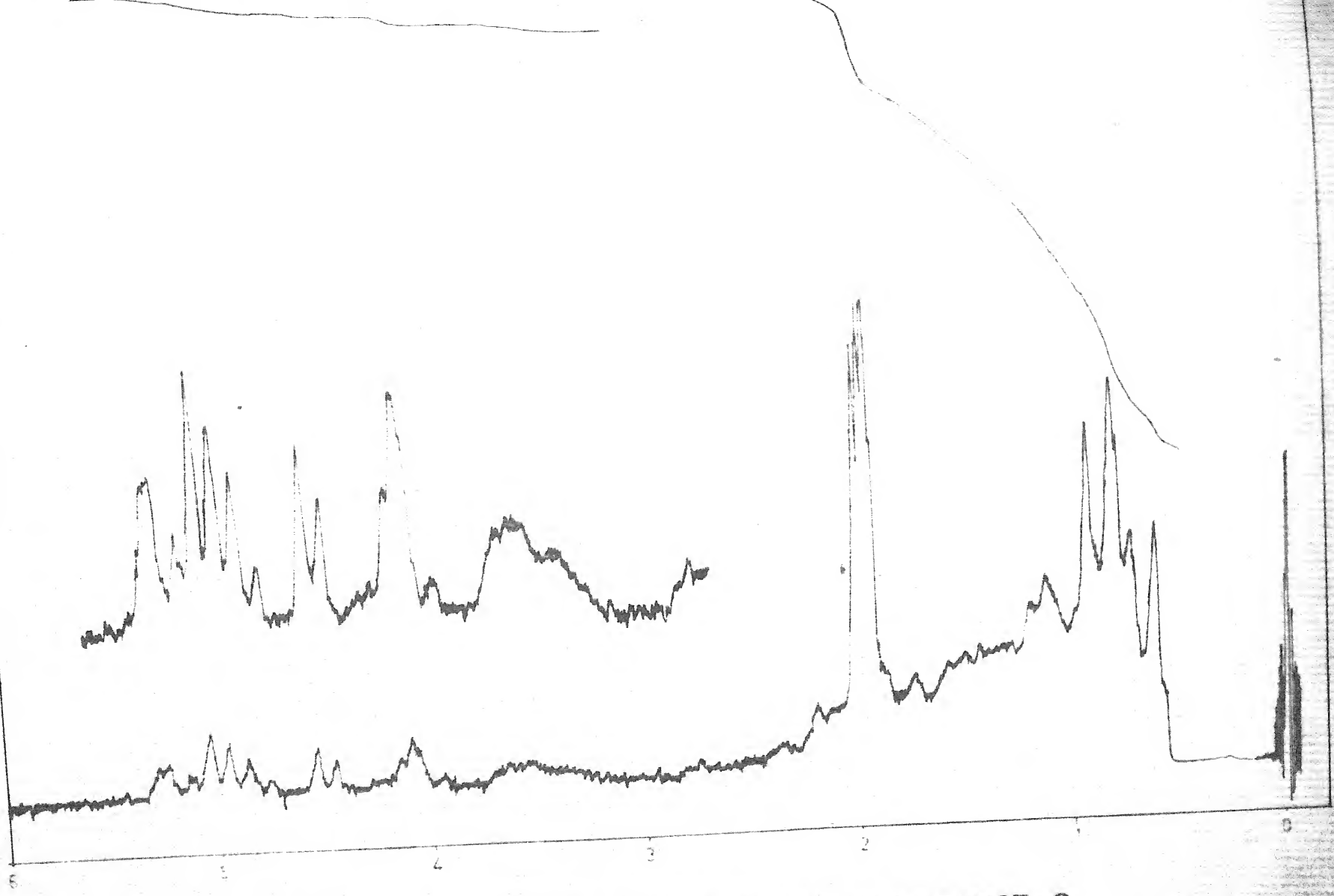


FIG-11 NMR SPECTRUM - ACETATE OF SUBSTANCE-C.

β-sitosterol by comparison (m.m.p., TLC, IR) with an authentic sample. Aqueous portion of the hydrolysate showed a single spot for sugar on paper chromatogram which was found to be identical with that of D-glucose. Therefore compound C was characterised as β-sitosterol-β-D-glucoside[94-95] (16) and this was confirmed by comparison of the m.p., TLC and IR with the authentic sample.

Substance D: This compound was isolated as crystalline solid, which crystallised from methanol as white octahedral prisms, m.p. 187°, $(\alpha)_D^{22^\circ}$ +67°. It is sparingly soluble in methanol, ethanol, acetone but freely soluble in water. A broad peak at 3380 cm^{-1} and sharp peak at 3330 and 1080 cm^{-1} in the IR spectrum of the compound D showed the presence of hydroxy function. It was analysed for $C_7H_{14}O_6$ and the molecular weight was confirmed by mass spectrometry m/z 195 $(M+H)^+$. It failed to decolourise Fehling's solution indicating it to be a non-reducing sugar. It had a Rf value of 0.49 on paper chromatogram developed in acetone: water (85:15, v/v). Finally, its identity as pinitol[77] (17) was confirmed by mixed melting point determination, co-paper chromatography, recording of super-imposable IR spectra with an authentic sample.

Substance E: It was obtained as an almost colourless solid which on repeated crystallisation from methanol yielded colourless needles m.p. 218-19° and was optically inactive. The compound was sparingly soluble in ethanol and methanol

but fairly soluble in water. The IR spectrum (Fig.12) displayed a broad peak at 3420 and 1060 cm^{-1} indicating the presence of hydroxyl group. It was analysed for $C_6H_{12}O_6$ and confirmed by FD mass spectrum m/z 181$(M+H)^+$ (Fig.13). It failed to give Fehling's test[75] suggesting it to be a non-reducing sugar. The substance E formed a hexa acetate having m.p. 215°. The IR spectrum of this derivative showed band at 1730 cm^{-1} for the acetoxy function. The identity of compound E with that of meso-inositol[96-97] (18) was established by ascending co-chromatography with an authentic sample and finally confirmed by mixed m.p. determination and comparison of the IR.

Free sugars: The remaining fraction after removal of the sugar alcohols (substance D and E) was chromatographed on Whatman No.1 filter paper using two different solvent system[81-82] i.e. n-butanol-acetic acid-water(4:1:5, v/v,upper) and ethylacetate-acetic acid-water(3:1:3, v/v) and aniline hydrogen phthalate as a spraying agent[83]. The identity of glucose, arabinose and sucrose were established on the basis of identical Rf values with those of known sugar samples.

Free amino acids: The characterisation of free essential (leucine, isoleucine, threonine, methionine, tryptophan) and non-essential (glutamic acid, alanine, serine, aspartic acid, glycine, histidine) amino acids were

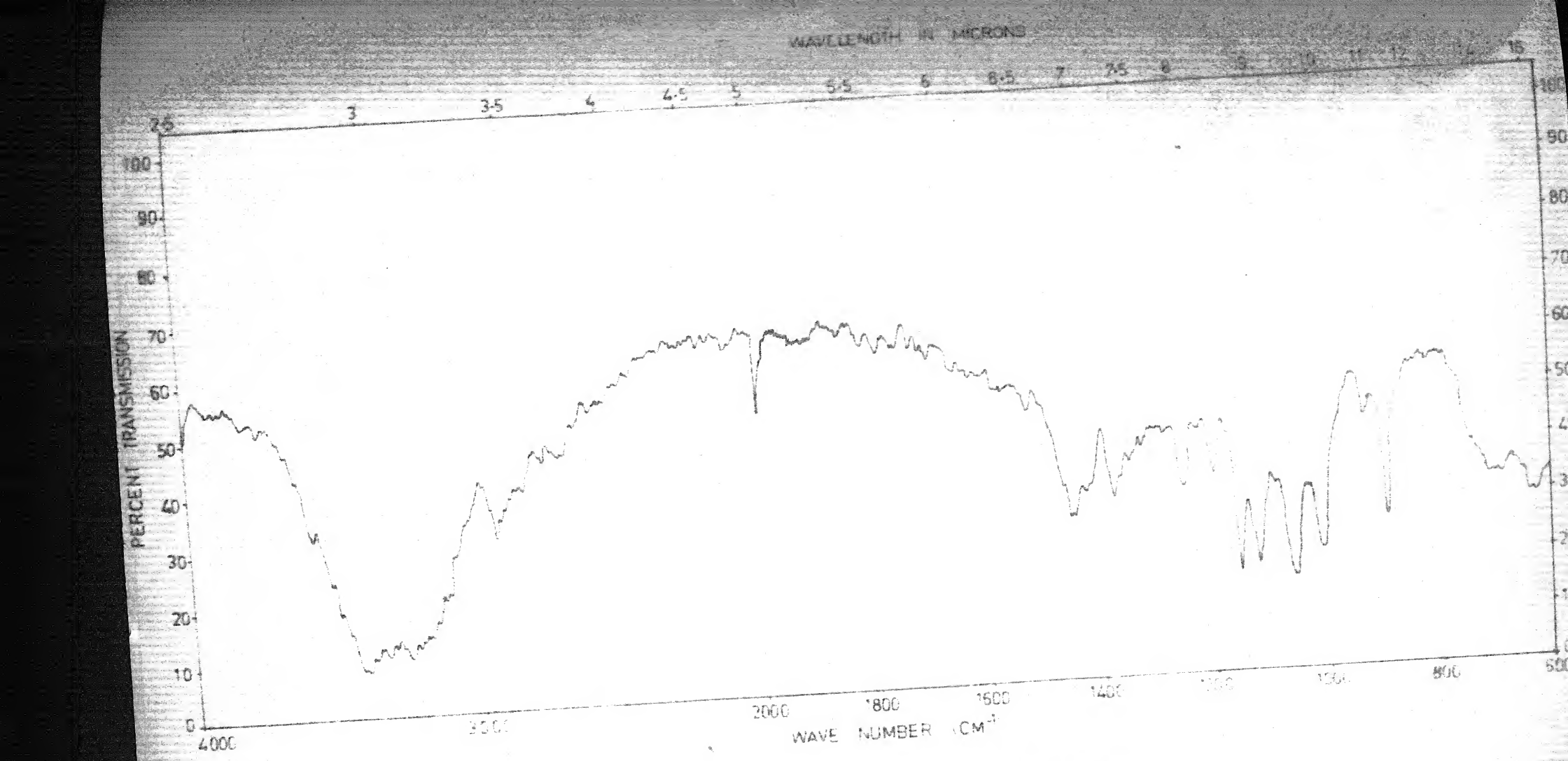


FIG-12 IR SPECTRUM OF SUBSTANCE-E

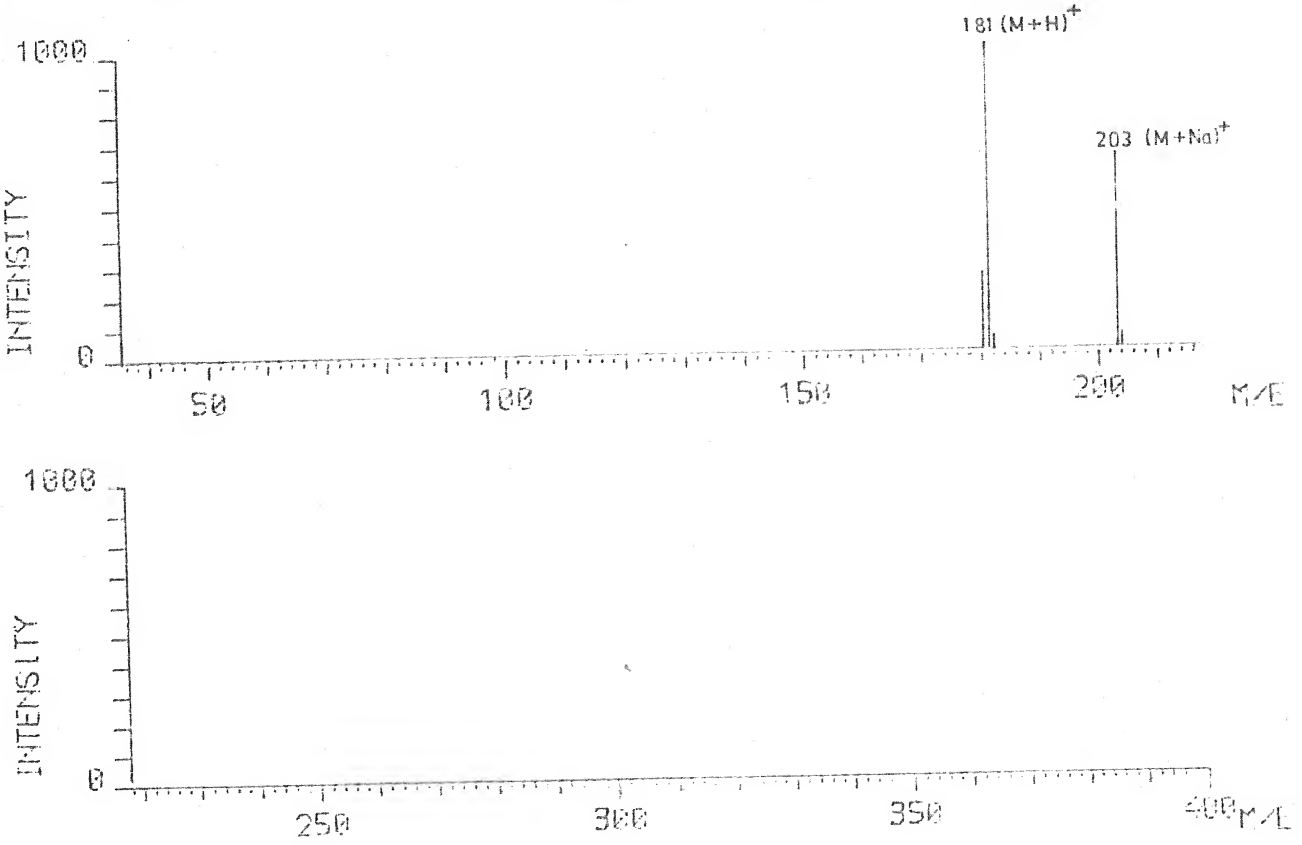


FIG-13 FD MASS SPECTRUM OF SUBSTANCE-E.

established by descending two dimensional paper chromatography[98-99] and compared with reference samples on Whatman paper No.1. The paper was run under two solvent system[100-101] i.e. (n-butanol-acetic acid-water, 4:1:5, v/v, upper) and (n-butanol-formic acid-water, 12:1:1, v/v, upper) and ninhydrin[102] as a spraying agent. The identity was confirmed on the basis of identical Rf values.

STRUCTURE OF THE COMPOUNDS

$Me(CH_2)_{25}Me$ (1)

$Me(CH_2)_{26}Me$ (2)

$Me(CH_2)_{27}Me$ (3)

$Me(CH_2)_{28}Me$ (4)

$Me(CH_2)_{29}Me$ (5)

$Me(CH_2)_{30}Me$ (6)

$Me(CH_2)_{31}Me$ (7)

$Me(CH_2)_{33}Me$ (8)

$Me(CH_2)_{32}Me$ (9)

$Me(CH_2)_{26}CH_2OH$ (10)

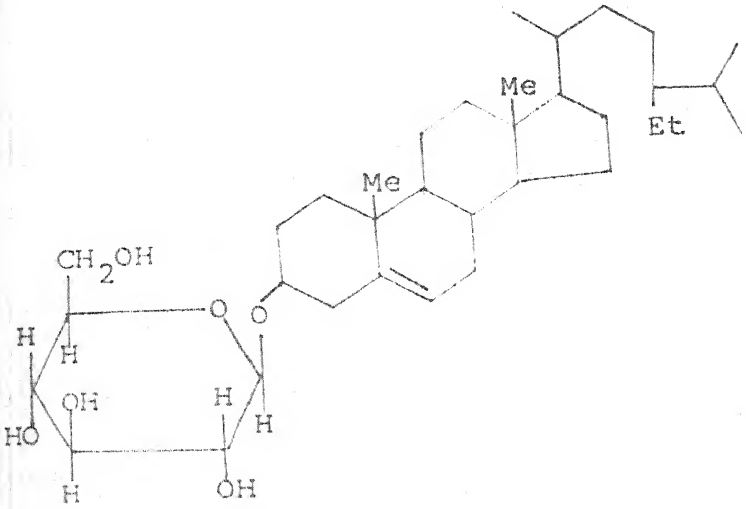
$Me(CH_2)_{27}CH_2OH$ (11)

$Me(CH_2)_{28}CH_2OH$ (12)

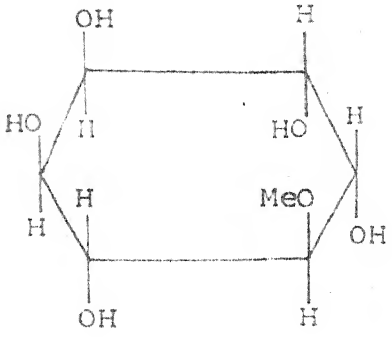
$Me(CH_2)_{30}CH_2OH$ (13)

$Me(CH_2)_{31}CH_2OH$ (14)

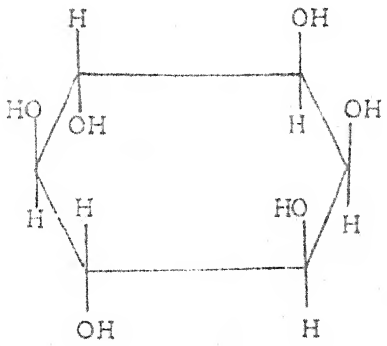
$Me(CH_2)_{32}CH_2OH$ (15)


Me
Et
Me
CH2OH
O
O
H
H
OH
H
HO
H
H
OH

(16)


OH
H
HO
H
HO
H
H
H
MeO
OH
OH
H

(17)


H
OH
HO
OH
H
OH
H
H
HO
H
OH
H

(18)

EXPERIMENTAL

ISOLATION OF CONSTITUENTS:

The plant was collected from the Institute campus during August and September and contained crude protein 11.1%, calcium 1.36% and phosphorus 0.09%. Air dried powdered plant material (2 kg) was exhaustively extracted with ethanol (95%, 4x6 lit.) by cold percolation. The ethanolic extract was evaporated under vacuum below 50°. The residual mass (100 g) so obtained was extracted successively with hexane (4x1 lit.), benzene (4x500 ml), ethyl acetate (4x500 ml) and acetone (4x500 ml) and on concentration afforded the following fractions.

1) Hexane soluble(30 g).
2) Benzene soluble (10 g).
3) Ethyl acetate soluble (5 g).
4) Acetone soluble (8 g).
5) Remaining extract (30 g).

COLUMN CHROMATOGRAPHY OF THE HEXANE FRACTION:

The dark green coloured hexane fraction was found to be a complex mixture on TLC. A part of it (20 g) was subjected to column chromatography over silica gel (400 g) in hexane. The elution was effected with hexane, benzene, ethyl acetate, ethanol and their mixture to afford forty fractions of 500 ml each. The fractions were combined on the basis of TLC and the results are shown in Table-3.

Table-3 Column chromatography of the hexane fraction (20 g)

Fr. No.	Eluant	Weight (g)	Remarks
1-3	Hexane	1.60	Contained substance A
4-8	Hexane:Benzene (80:20)	0.37	Contained substance B
9-11	Hexane:Benzene (50:50)	0.80	-do-
12-16	Benzene	0.40	-do-
17-22	Benzene:Ethyl acetate (50:50)	0.10	Complex mixture
23-28	Benzene:Ethyl acetate (30:70)	0.20	-do-
29-34	Ethyl acetate	0.10	-do-
35-36	Ethyl acetate:Ethanol (50:50)	4.00	-do-
37-40	Ethanol	6.00	-do-

SUBSTANCE A:

The fractions 1-3 were combined and solvent evaporated off. The residue (1.6 g) was rechromatographed over a short column of neutral alumina. The eluant from benzene afforded substance A (100 mg), m.p. 66-67° which recrystallised from benzene.

IR) ν_{max}^{KBr}: 2900, 2850(-CH-stretching), 1466, 1375(-CH-bending), 740 and 720 cm^{-1}.

EIMS: m/z 464, 436, 422, 408, 394, 393, 380, 379, 365, 351, 337, 323, 309, 295, 281, 267, 253, 239, 225, 211, 197, 183, 169, 155, 141, 127, 113, 99, 85, 71, 57 and 43.

FIMS: m/z 492(M^+), 464(M^+), 450 (M^+), 436 (M^+), 422(M^+), 408 (M^+), 394 (M^+) and 380(M^+).

SUBSTANCE B:

The fractions 4-16 were mixed and the solvent removed. The residue (1.57 g) after TLC examination over silica gel was found to be a mixture of closely related compounds which were separated by PLC using benzene as a developing system to yield substance B (50 mg) and was crystallised from hexane. M.p. 78-84°.

$IR\nu_{max}^{KBr}$: 3400 (-CH-), 2920, 2850, 1450, 1375, 1040, 740 and 720 cm^{-1}.

EIMS: m/z 476, 448, 420, 406, 392, 378, 364, 349, 335, 321, 307, 293, 279, 265, 251, 237, 223, 209, 195, 181, 167, 153, 139, 125, 101, 87, 73, 59 and 45.

FDMS: m/z 494 (M^+, 7.90%), 480 (M^+, 9.88%) 4.78 (M^+, 6.71%), 466 (M^+, 13.8%), 450 (M^+, 8.30%), 438 (M^+, 10.67%), 436 (M^+, 11.85%), 424 (M^+, 6.71%), 422 (M^+, 8.30%), 410 (M^+, 5.92%) and 394 (M^+, 9.88%).

Acetylation of substance B:

A mixture of substance B (20 mg), acetic anhydride (1 ml) and dry pyridine (1 ml) was refluxed for 4 hrs on steam bath. The usual work up and crystallisation from chloroform yielded a white micro-crystalline product (22 mg). M.p. 68-72°.

IR ν_{max}^{KBr} : 2900, 2850, 1730 ($-OCOCH_3$), 1460, 1360, 1240, 740 and 720 cm^{-1}.

EIMS: m/z 536, 508, 480, 476, 448, 420, 406, 392, 378, 364, 350, 335, 321, 307, 292, 278, 264, 250, 236, 222, 208, 194, 180, 166, 152, 138, 124, 110, 97, 83, 71, 57 and 43.

CIMS: m/z 537, 535, 523, 521, 509, 507, 481, 479, 477, 467, 465, 463, 453, 451, 449, 421, 407 and 393.

FDMS: m/z 536(M^+), 522(M^+), 508(M^+), 480(M^+), 478(M^+), 466(M^+), 452(M^+), 450(M^+), 436(M^+), 422(M^+) and 394(M^+).

COLUMN CHROMATOGRAPHY OF BENZENE AND ETHYL ACETATE SOLUBLE FRACTIONS:

The benzene and ethyl acetate soluble fractions were found to have identical spots. Both of them were combined on the basis of TLC and was chromatographed on a column of silica gel (400 g) in benzene. The elution was performed by increasing proportions of ethyl acetate followed by ethanol. Fifty fractions of 250 ml each were collected and mixed on the basis of TLC and the results are presented in Table-4.

Table-4 Column chromatography of benzene and ethyl acetate soluble fractions (15 g).

Fr. No.	Eluant	Weight (g)	Remarks
1-4	Benzene	3.00	Oily residue
5-8	Benzene:Ethyl acetate (80:20)	0.40	Mixture
9-14	Benzene:Ethyl acetate (60:40)	0.50	-do-
15-22	Benzene:Ethyl acetate (40:60)	0.65	Substance C
23-30	Ethyl acetate	0.90	-do-
31-34	Ethyl acetate	0.20	-do-
35-42	Ethyl acetate:Ethanol (50:50)	2.00	Mixture
43-50	Ethanol	2.00	-do-

SUBSTANCE C: β-sitosterol-β-D-glucoside

The fractions 15-34 were mixed and the solvent removed. The residue (1.75 g) on crystallisation from methanol yielded substance C as colourless needles (400 mg). M.p. 300°, $(\alpha)_D^{25°}$ - 43° (pyridine).

IR ν_{max}^{KBr} : 3400, 2960, 2930, 2850, 1468, 1380, 1365, 1260, 1105, 1070, 1020, 960, 880, and 780 cm^{-1}.

Analysis: Found C, 72.20; H, 10.05

$C_{35}H_{60}O_6$ required C, 72.81; H, 10.48%

Acetylation of substance C:

50 (mg) of the substance C was dissolved in dry pyridine and acetic anhydride (2ml each). The mixture was refluxed for one hour and the solvent were removed under vacuum and the residue diluted with water. The white crystalline precipitate thus obtained was filtered and recrystallised from ethanol. M.p. 168-170°.

IR ν_{max}^{KBr} : 2960, 2850, 1730, 1240, 1220, 1160, 1105, 1070 and 880 cm^{-1}.

NMR ($CDCl_3$): δ 0.5-1.5 (s, 18H, 6xCH_3), 1.94, 1.95, 1.97, 1.99 (s, 3Hx4x$OCOCH_3$), 3.58 (m, 1H, -C<u>H</u>OH), 4.1 (m, 2H, C-1'<u>H</u>, 4.5 (m, 1H, C-2'<u>H</u>),

4.7-5.1(m, 4H, C-3'H, C-4'H, C-5'H and C-6'H) and 5.26(m, 1H, -C=CH-CH_2-).

EIMS: m/z 744(M^+), 684, 624, 564, 504, 331 and 169.

CIMS: m/z 762$(M+NH_4)^+$, 397 and 366.

Analysis: Found C, 69.18 ; H, 9.47

$C_{43}H_{68}O_{10}$ required C, 69.35 ; H, 9.13%

Acid hydrolysis of substance C:

Compound C (100 mg) was refluxed with ethanolic H_2SO_4 (5%) for 6 hrs. Ethanol was distilled off under reduced pressure and the residue diluted with water. The reaction mixture was extracted with ethyl acetate and washed with water. Ethyl acetate extract was dried over anhydrous sodium sulphate and then concentrated. The aglycone thus obtained was crystallised from benzene-methanol to afford colourless crystals (45 mg). M.p. 134-135°, $(\alpha)_D^{25°}$ - 40°(c, 1, $CHCl_3$).

IR ν_{max}^{KBr} : 3240, 2910, 2825, 1655, 1450, 1370, 1360, 1060, 970, 850 and 810 cm^{-1}.

Analysis: Found C, 84.36 ; H, 12.24

$C_{29}H_{50}O$ required C, 84.06 ; H, 12.07%

Identification of glucose from substance C:

The aqueous portion was neutralised by barium carbonate and concentrated. Paper chromatography of the

concentrate was done alongwith authentic sample of D-glucose, galactose, and rhamnose in n-butanol-acetic acid-water (4:1:5, v/v, upper) solvent system. The spots were visualised by spraying the chromatogram with aniline hydrogen phthalate reagent and dried in an oven at 105°. The concentrate showed a single spot and it compared well with that of an authentic sample of D-glucose. Hence the carbohydrate unit of the glycoside was confirmed as D-glucose.

ACETONE SOLUBLE FRACTION:

SUBSTANCE D: D(+) pinitol

The acetone extract (8 g) was concentrated under reduced pressure to 100 ml and kept in refrigerator for a week. A crystalline solid deposited which on crystallisation from methanol yielded substance D (4.5 g) as while octahedral prisms. M.p. 186-187°, $(\alpha)_D^{37^\circ}$ +67 (H_2O).

IR ν_{max}^{KBr}:	3380, 3330, 2920, 2880, 1470, 1440, 1370, 1330, 1270, 1240, 1120, 1080, 1040, 1010, 990, 950, 895, 855 and 750 cm^{-1}.
FDMS:	m/z 195 $(M+H)^+$.
Analysis:	Found C, 43.7 ; H, 7.48
$C_7H_{14}O_6$	required C, 43.35; H, 7.21%

COLUMN CHROMATOGRAPHY OF THE REMAINING EXTRACT:

The remaining extract (30 g) left after the extraction with hexane, benzene, ethyl acetate and acetone was found to be a mixture of sugars. A part of it (12 g) was chromatographed over a column of silica gel (350 g). The elution was carried out with chloroform and by adding increasing proportion of methanol. The fractions (500 ml each) were combined on the basis of TLC and the results are recorded in Table-5.

Table-5 Column chromatography of the remaining extract (12 g)

Fr. No.	Eluant	Weight (g)	Remarks
1	Chloroform	-	No residue
2	Chloroform:Methanol (90:10)	0.40	Mixture
3	Chloroform:Methanol (80:20)	3.50	Contained substance D
4-6	Chloroform:Methanol (60:40)	0.40	Mixture
7-8	Chloroform:Methanol (40:60)	2.00	Contained substance E
9-10	Chloroform:Methanol (20:80)	2.00	Mixture containing free sugar
11-13	Methanol	1.25	-do-

Fraction 3 on keeping in a refrigerator deposited a crystalline solid which on repeated crystallisation from methanol after charcoal treatment yielded substance D(1.4 g).

SUBSTANCE E: Meso-inositol

The fractions 7-8 on solvent fractionation and crystallisation from methanol furnished colourless crystals of substance E (76 mg). M.p. 218-19°.

IR ν_{max}^{KBr}:	3420 (OH), 2940, 2880, 1470, 1450, 1380, 1370, 1060(OH), 900 and 810 cm^{-1}.
FDMS:	m/z 203$(M+Na)^+$ and 181$(M+H)^+$.
Analysis:	Found C, 40.30 ; H, 6.75
$C_6H_{12}O_6$	required C, 40.0 ; H, 6.66%

Acetylation of substance E:

A mixture of compound E (10 mg), dry pyridine and acetic anhydride (0.5 ml each) was refluxed on an oil bath at 120-30° for 4 hrs. The usual work up furnished a white solid (8 mg) which was crystallised from methanol. M.p. 215°.

IR ν_{max}^{KBr}:	1730, 1370, 1210, 1060, 920 and 740 cm^{-1}.

IDENTIFICATION OF FREE SUGARS:

The fractions 9-13 obtained after eluting the column with chloroform-methanol (20:80) and methanol was concentrated under reduced pressure to give a brown syrupy product. This was chromatographed on Whatman filter paper No.1 alongwith the authentic samples of sugars.

The chromatograms were developed in two different solvents i.e. n-butanol-acetic acid-water (4:1:5, v/v, upper) and ethyl acetate-acetic acid-water (3:1:3, v/v, upper) and run for 14 hrs by the descending technique. The spots were visualised by spraying the chromatograms with aniline hydrogen phthalate reagent and dried in an oven at 105°. The fractions 9-13 showed the presence of three spots having identical Rf values with that of D-glucose, arabinose and sucrose respectively.

IDENTIFICATION OF FREE AMINO ACIDS:

The defatted (with hexane) plant material was extracted with aqueous ethanol (7:3) at its boiling temperature and concentrated under vacuum. A dark brown syrupy liquid was obtained which showed the presence of amino acids. The concentrated residue was extracted with chloroform to remove colouring matter and the free amino acid were taken in n-butanol saturated with water. Amino acids were detected by two dimensional descending paper chromatography on Whatmann No.1 filter paper. The chromatograms were run in two different solvent system i.e. n-butanol-acetic acid-water (4:1:5, v/v, upper) and n-butanol-formic acid-water (12:1:1, v/v, upper). After 16 hrs of irrigation the chromatograms were dried, sprayed with ninhydrin (0.1% acetone) and heated in an oven for 5 minutes at 80°. The spots so produced indicated the

presence of free essential (leucine, isoleucine, threonine, methionine, tryptophan) and non-essential amino acids (glutamic acid, alanine, serine, aspartic acid, glycine, histidine). The identity of above amino acids were confirmed by co-chromatography with known reference amino acids run simultaneously under identical conditions.

CHAPTER-III

THE CHEMICAL INVESTIGATION OF

LINDENBERGIA URTICAEFOLIA LEHM.

INTRODUCTION:

Lindenbergia urticaefolia Lehm. Syn. L.indica Linn. (Scrophulariaceae) known as Bhintachati in Gujarati and Dhol in Marathi.

Distribution:

Throughout India, on walls and banks, ascending to 2000 m in the Himalayas from Jammu to Nilgiris. It is also distributed in Burma and Afghanistan.

Botanical description and uses:

It is an erect or creeping, glandular hairy annual herb, 10-30 cm in height; leaves short, petioled, broadly ovate, crenate, serrate, gland-villous on both sides; flowers shortly pedicelled, solitary or in pairs in the axils of large leaves, sometimes forming axillary or terminal spicate racemes; calyx densely gland-villous; corollas yellow, spotted with red or purpled lipped; ovary pubescent round the apex; capsule oblong, hairy above.

The plant possess a faint aromatic odour and a slightly bitter teste. Its juice is given in chronic bronchitis[103-104] and mixed with that of coriander applied to skin erruption. The plant also possess hypotensive activity[105].

PREVIOUS WORK:

The literature survey has revealed that Tiwari and Choudhary[106-107] have reported two steryl glycosides i.e. α-L-rhamnopyranosyl (1→4) β-D-glucopyranosyl

Lindenbergia urticaefolia

(1→3) sitosterol, α-L-rhamnopyranosyl (1→5) α-L-arabinofuranosyl (1→3) sitosterol, oleanolic acid, 7-hydroxy flavone, quercetin and friedelan-3-β-ol from whole plant.

PRESENT WORK:

The plant occurs as an annual herb in Bundelkhand and adjoining areas during monsoon. In view of its wide occurrence the investigations on the chemical constituents of the plant were undertaken to assess its biological value, toxic nature as well as its potential for utilisation as livestock feed.

The crude ethanolic extract of the whole plant of L.urticaefolia was fractionated in the usual way into hexane, benzene and acetone soluble fractions. Hexane and acetone soluble fractions were investigated in detail. The column chromatography of these fractions over silica gel followed by preparative TLC furnished following substances as mentioned in Table-1.

Table-I

Sl. No.	Name of Constituents	Molecular formula	m.p.
1.	Substance A		65-67°
	a) Heptacosane	$C_{27}H_{56}$	
	b) Octacosane	$C_{28}H_{58}$	
	c) Nonacosane	$C_{29}H_{60}$	
	d) Triacontane	$C_{30}H_{62}$	
	e) Hentriacontane	$C_{31}H_{64}$	
	f) Dotriacontane	$C_{32}H_{66}$	
	g) Tritriacontane	$C_{33}H_{68}$	
2.	Substance B β-sitosterol palmitate	$C_{45}H_{80}O_2$	80-83°
3.	Substance C β-sitosterol	$C_{29}H_{50}O$	136-37°
4.	Substance D Unidentified	-	146°
5.	Substance E β-sitosterol-β-D-glucoside	$C_{35}H_{60}O_6$	296-98°
6.	Substance F Mannitol	$C_6H_{14}O_6$	166-67°
7.	Substance G Apigenin	$C_{15}H_{10}O_5$	343-44°
8.	Free Sugars		
	a) Glucose		
	b) Arabinose		
	c) Rhamnose		

RESULTS AND DISCUSSION

Substance A: m.p. 65-67°. Crystallised from ethyl acetate as colourless waxy solid. The IR spectrum revealed its aliphatic nature with the possibility of it being a straight chain saturated hydrocarbon[52]. However, GLC analysis (Fig.1) showed it to be a mixture of mainly seven components. The electron impact and field ionisation mass spectral studies (Fig. 2 and 3) showed substance A to be a mixture of the following hydrocarbons[53-57] i.e. heptacosane (1), octacosane (2), nonacosane (3), triacontane (4), hentriacontane (5), dotriacontane (6) and tritriacontane (7). The approximate percentage composition of the mixture as found from GLC and FIMS data is presented in Table-2.

Table-2

Peak No.	GLC DATA %	FIMS DATA %	FIMS DATA Molecular Wt.
1	14.7	6.7	380
2	7.6	9.1	394
3	25.9	23.5	408
4	7.2	8.4	422
5	29.3	33.6	436
6	4.3	7.0	450
7	10.8	11.4	464

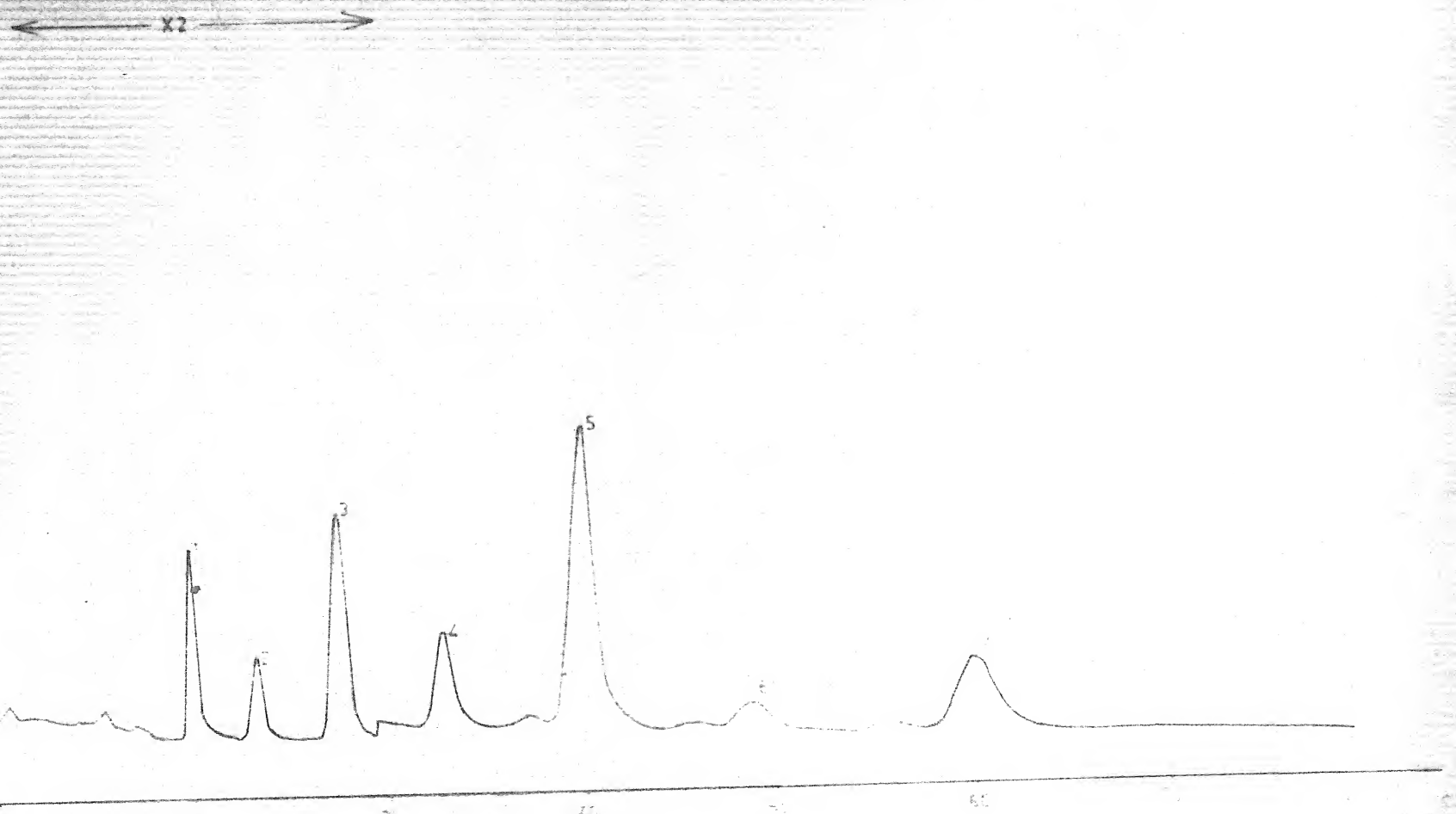


FIG-1 GLC OF SUBSTANCE-A

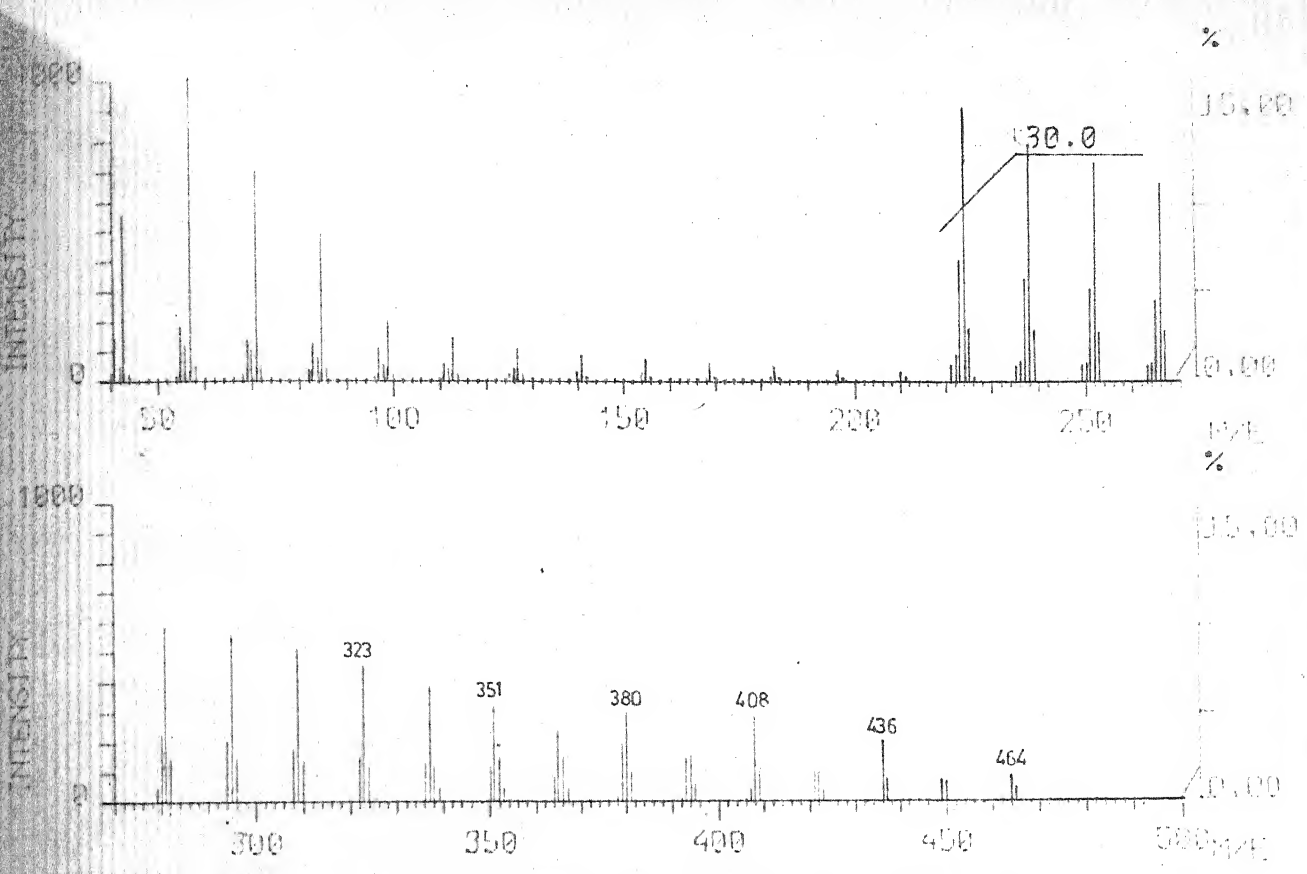


FIG-2 EI MASS SPECTRUM OF SUBSTANCE-A.

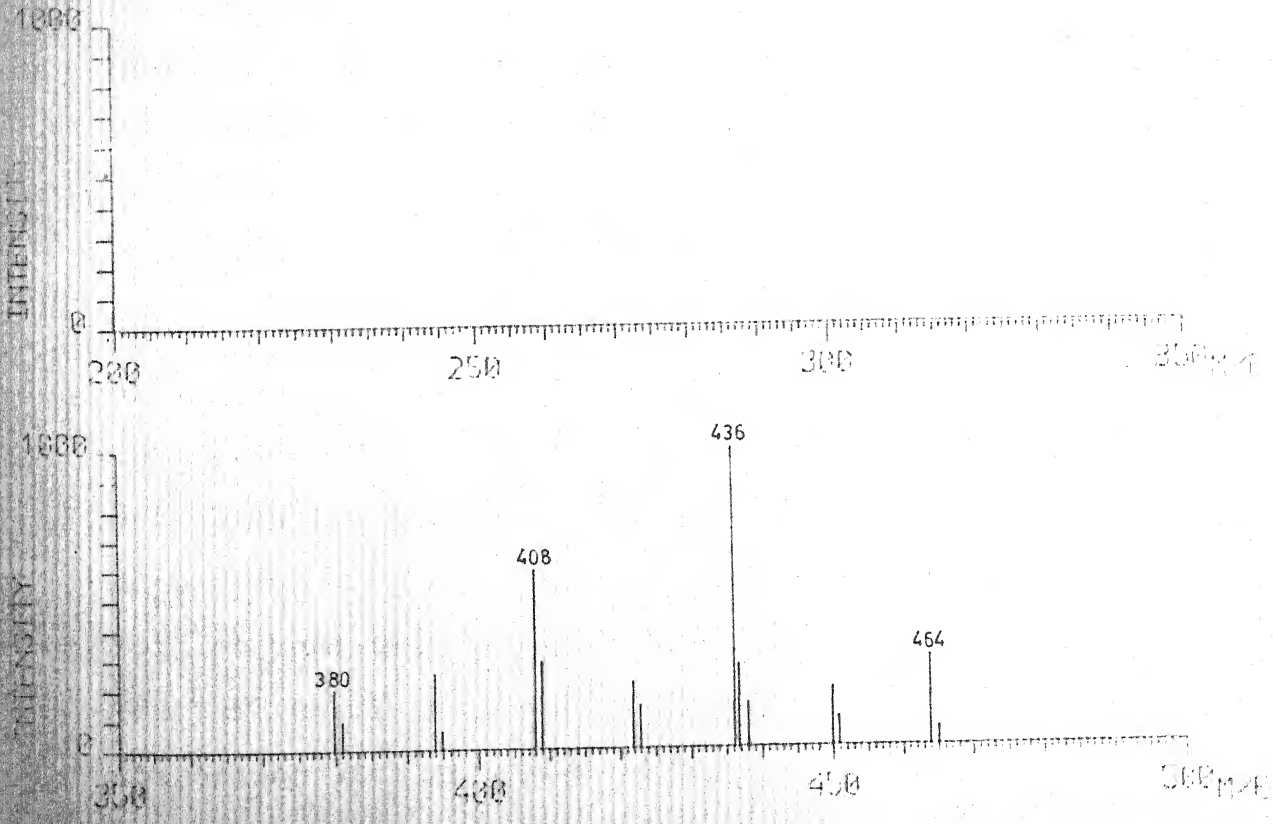


FIG-3 FI MASS SPECTRUM OF SUBSTANCE-A.

Substance B: m.p. 80-83°. Crystallised from hexane as colourless needles. The IR spectrum (Fig.4) showed an intense band at 1750 cm^{-1} indicating the presence of a carbonyl group. The electron impact mass spectrum showed prominent even mass ion peaks at m/z 396, 638 and 652. The peaks at m/z 57, 71, 83, 85, 97, 111 etc. suggested the presence of an aliphatic chain in the molecule and the peaks at m/z 255 and 396 were indicative of a steroid nucleus. The chemical ionisation (CH_4) mass spectrum of substance B showed peaks at m/z 257, 397, 639 and 653. From the CIMS data it was clear that the molecular weight of the major component in substance B was 652. This was further confirmed by field desorption mass spectrum (Fig.5) which showed a peak at m/z 652. These observations suggest that the major component in substance B are aliphatic acid (m.wt. 256) and a sterol (m. wt.414). This was further confirmed by the ammonia chemical ionisation mass spectra (Fig.6) of substance B which gave peaks at m/z 670 $(M+NH_4)^+$, 397 $(sterol-H_2O+H)^+$ and 274 $(acid+NH_4)^+$. The presence of sterol was also indicated by Liebermann-Burchard test[61]. Alkaline hydrolysis of substance B afforded a product characterised as β-sitosterol, m.p. 135°(Chloroform-methanol), m/z 414(M^+). Its identity was further confirmed by direct comparison with an authentic sample. FDMS of the acid portion confirmed the molecular weight of the acid as 256. The acid was identified as palmitic acid by direct comparison with an authentic sample. Hence substance B was

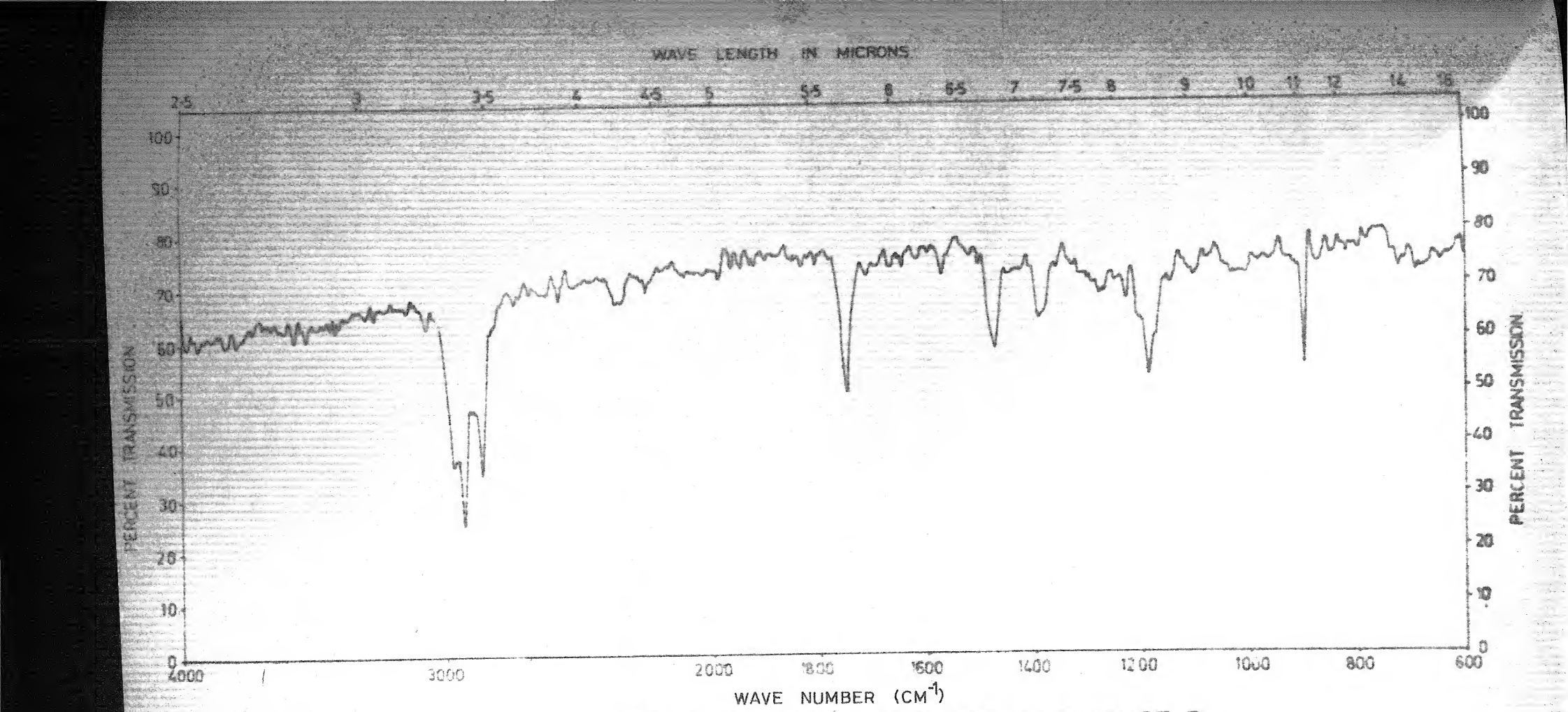


FIG-4 IR SPECTRUM OF SUBSTANCE-B.

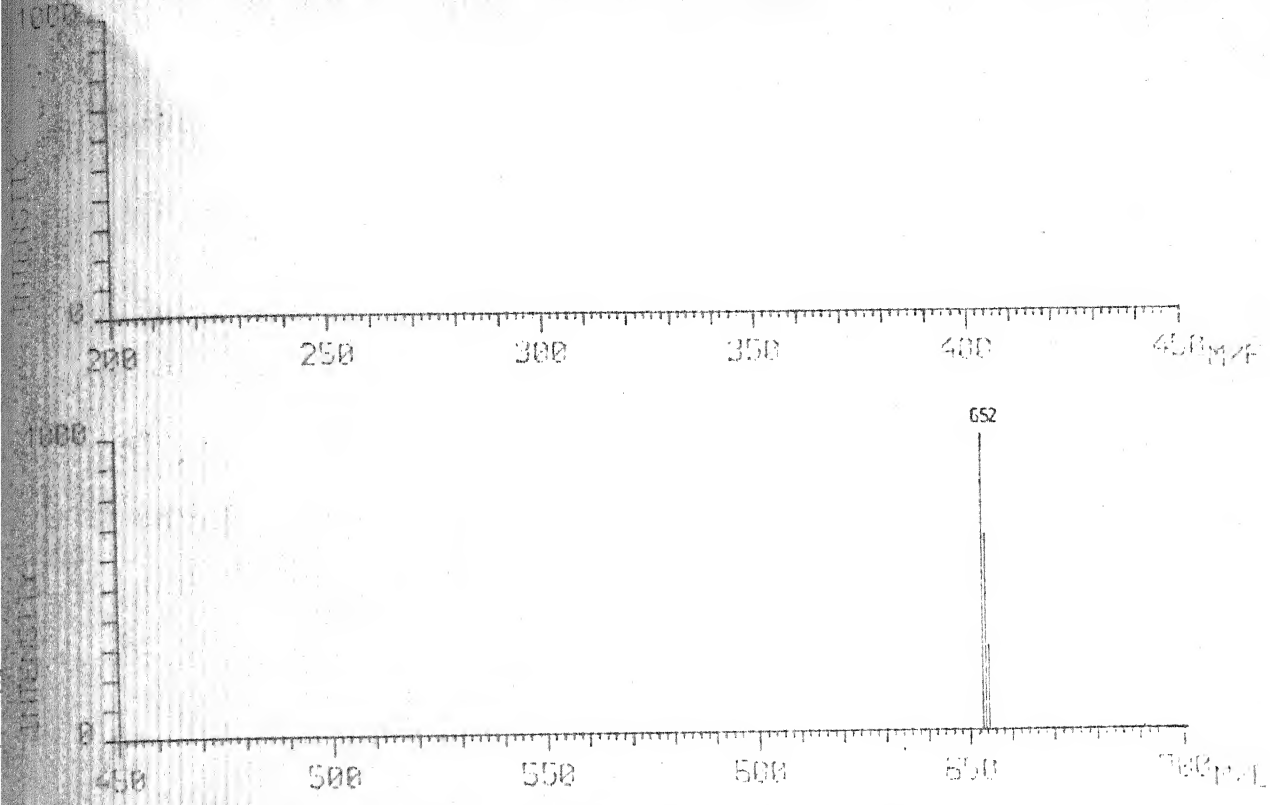


FIG-5 FD MASS SPECTRUM OF SUBSTANCE-B.

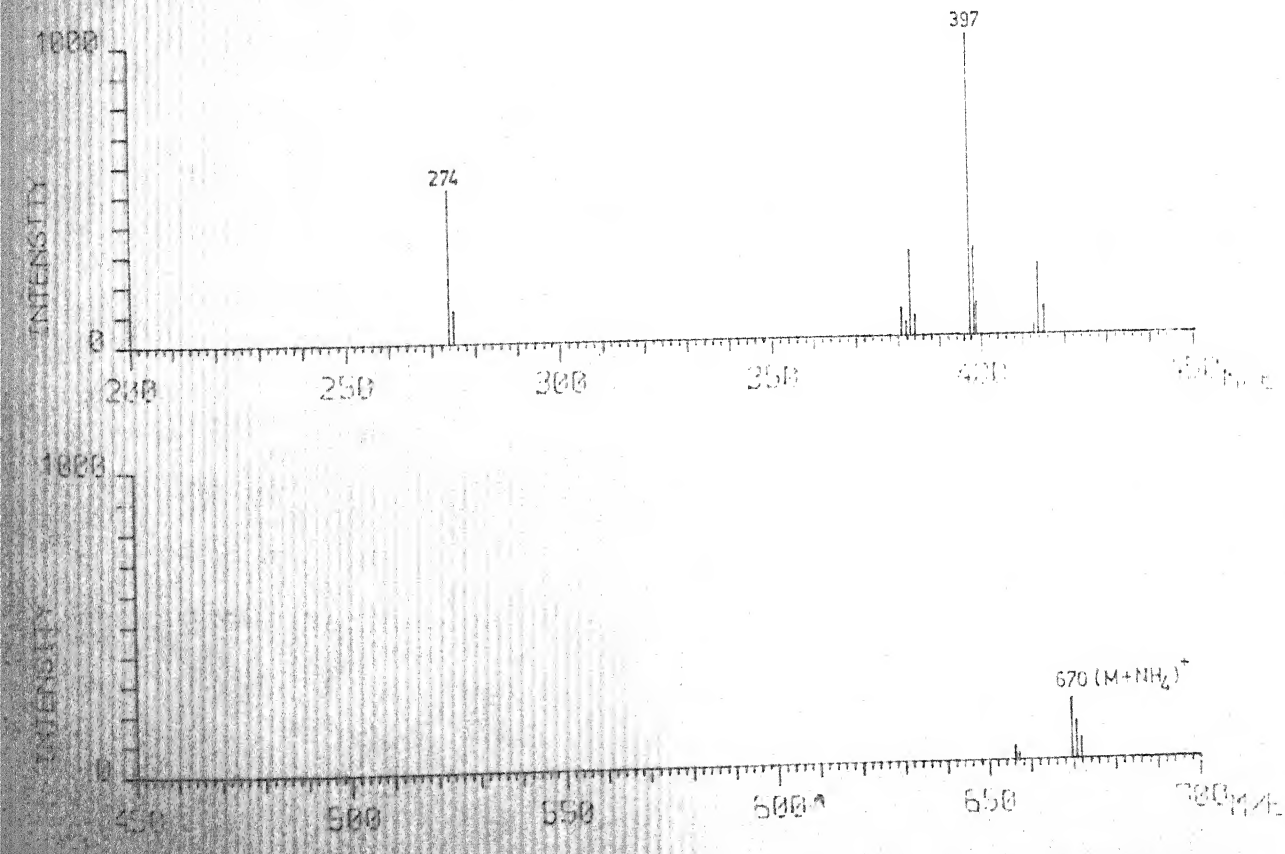


FIG-6 CI MASS SPECTRUM (NH_3) OF SUBSTANCE-B.

thus identified as β-sitosterol palmitate[108] (8) and its physico-chemical data corresponded the reported values in the literature.

Substance C: m.p. 136-37°, $(\alpha)_D^{25°}$ -40° (c.1, $CHCl_3$) was assigned the molecular formula $C_{29}H_{50}O$ on the basis of its micro analysis and mass spectral data m/z 414(M^+). It gave a sequence of colours (pink-violet-blue green) under Liebermann-Burchard test[61] indicating it to be a sterol derivative. The IR and NMR spectrum of the compound led to its identification as β-sitosterol[63] (9) which was confirmed by comparison with an authentic sample.

Substance D: This compound was obtained as solid mass which on crystallisation from benzene-methanol (80:20) yielded a colourless solid m.p. 146°. Sharp peaks occurring in the IR spectrum at 3360 and 1640 cm^{-1} indicated the presence of hydroxyl and carbonyl function in the molecule. However, HPLC analysis showed it to be a mixture of mainly four components. The electron impact mass spectrum showed prominent molecular ion peaks at m/z 620, 648, 662 and 676 which was also observed in the field ionisation mass spectrum, hence the identity of the molecular ion peaks seems to be confirmed. However, since the mixture could not be resolved further studies were not carried out.

Substance E: It was obtained as colourless needles after repeated crystallisation from methanol, m.p. 296-98°, $(\alpha)_D^{25°}$ - 43° (pyridine). Elemental analysis corresponded to a molecular formula $C_{35}H_{60}O_6$. Peaks at 3400 and 1020 cm^{-1} in the IR spectrum of the substance showed the presence of hydroxyl group and glycosidic linkage respectively. It gave positive Liebermann-Burchard test[61] for sterols and also responded positively to Fiegel test[65]. These two tests considered together led to the conclusion that this compound is a steroidal glycoside. It was found to be identical with β-sitosterol-β-D-glucoside[94-95] (10) by comparison of its IR, co-TLC and mixed melting point determination with an authentic sample.

Substance F: Crystallised from ethanol as colourless shining needles, m.p. 166-67°. It was sparingly soluble in ethanol and methanol. It was analysed for $C_6H_{14}O_6$. The IR spectrum (Fig.7) displayed a broad peak at 3420 and a sharp peak at 1080 cm^{-1} indicative of the presence of hydroxyl function. The FD mass spectrum (Fig.8) showed molecular ion peak at m/z 183 $(M+H)^+$. The presence of peaks at m/z 61, 91 etc suggested it to be a sugar derivative. It failed to decolorise Fehling's solution[75] suggesting it to be a non-reducing sugar. Acetylation of substance F furnished a hexaacetate[109] as confirmed by mass spectrometry m/z 434 (M^+). m.p. 120°. Its IR spectrum (Fig.9) showed the absence of hydroxyl function

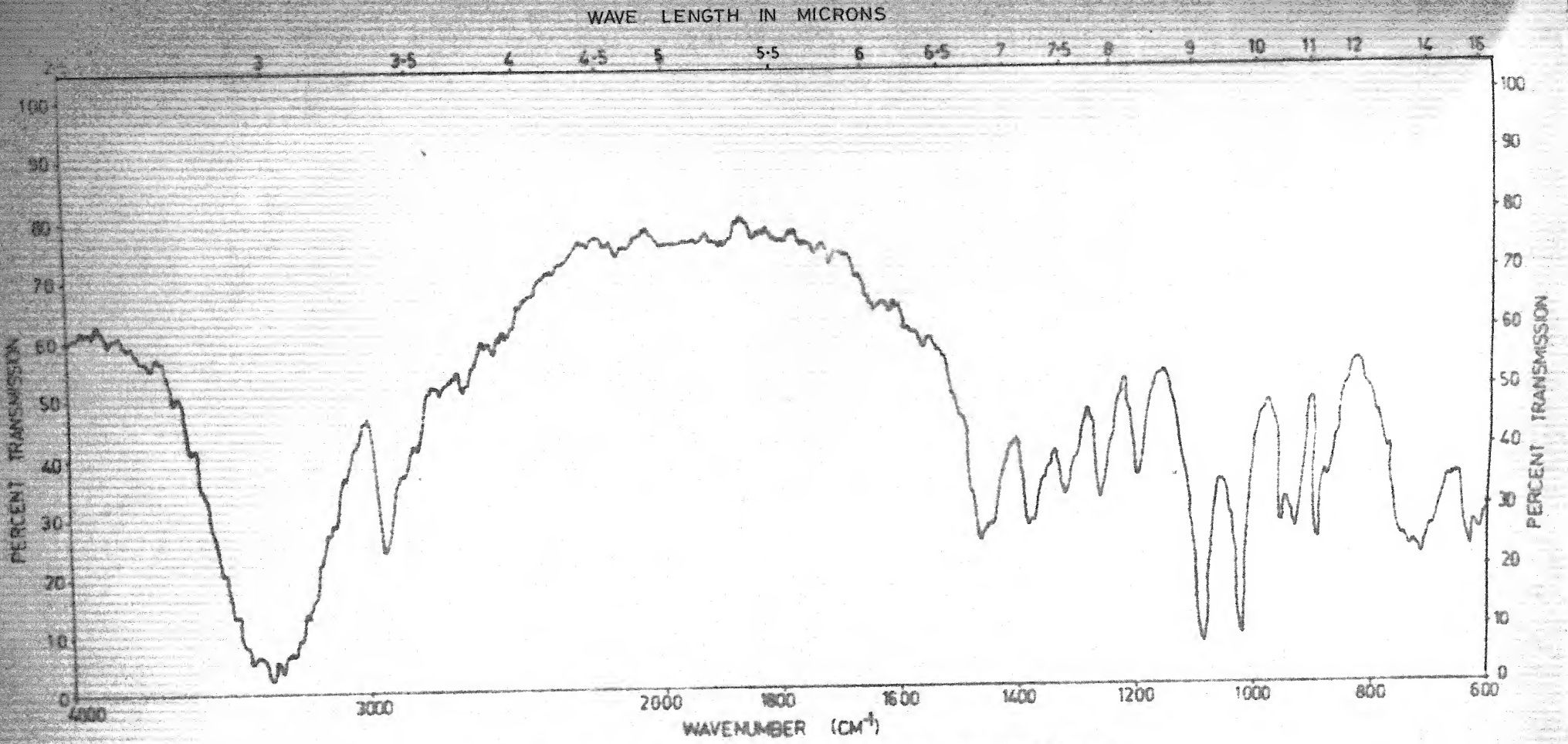


FIG-7 IR SPECTRUM OF SUBSTANCE-F.

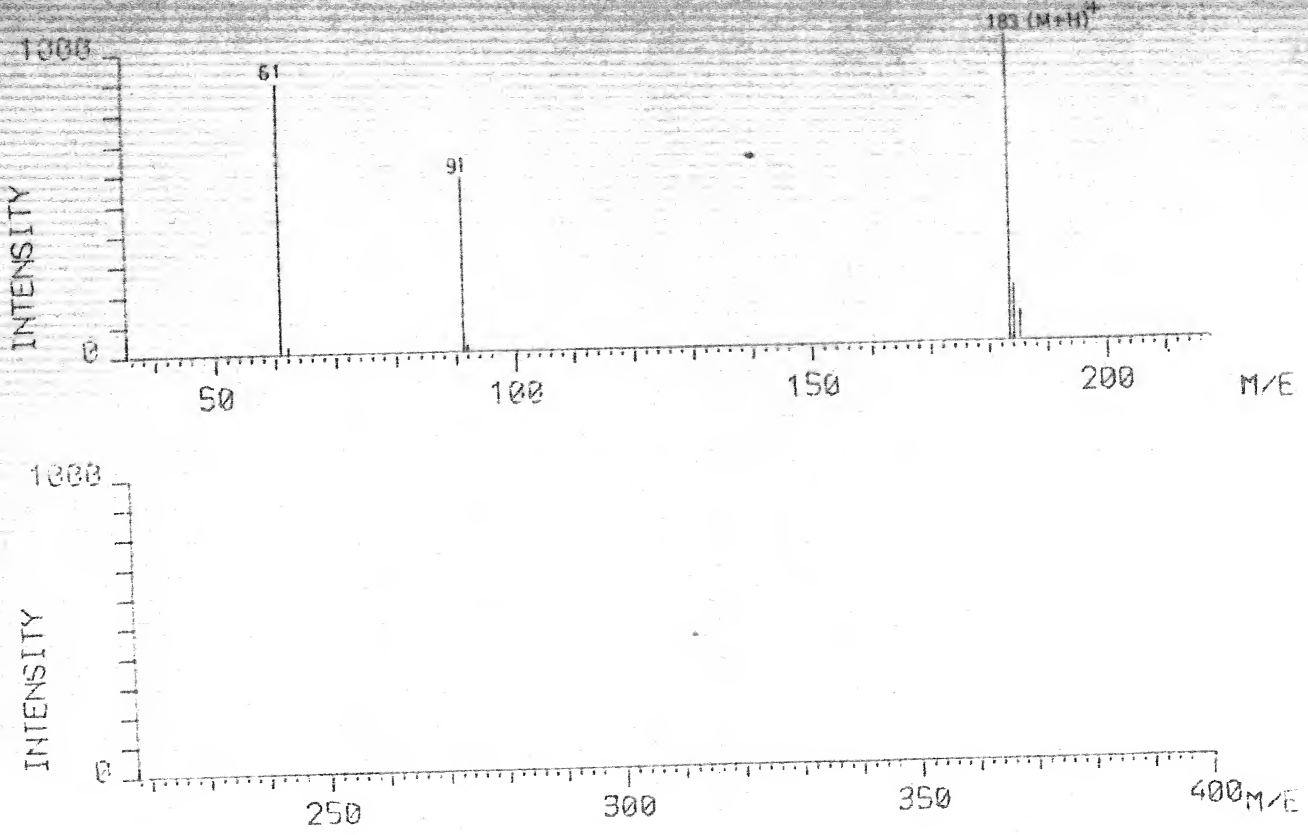


FIG-8 FD MASS SPECTRUM OF SUBSTANCE-F.

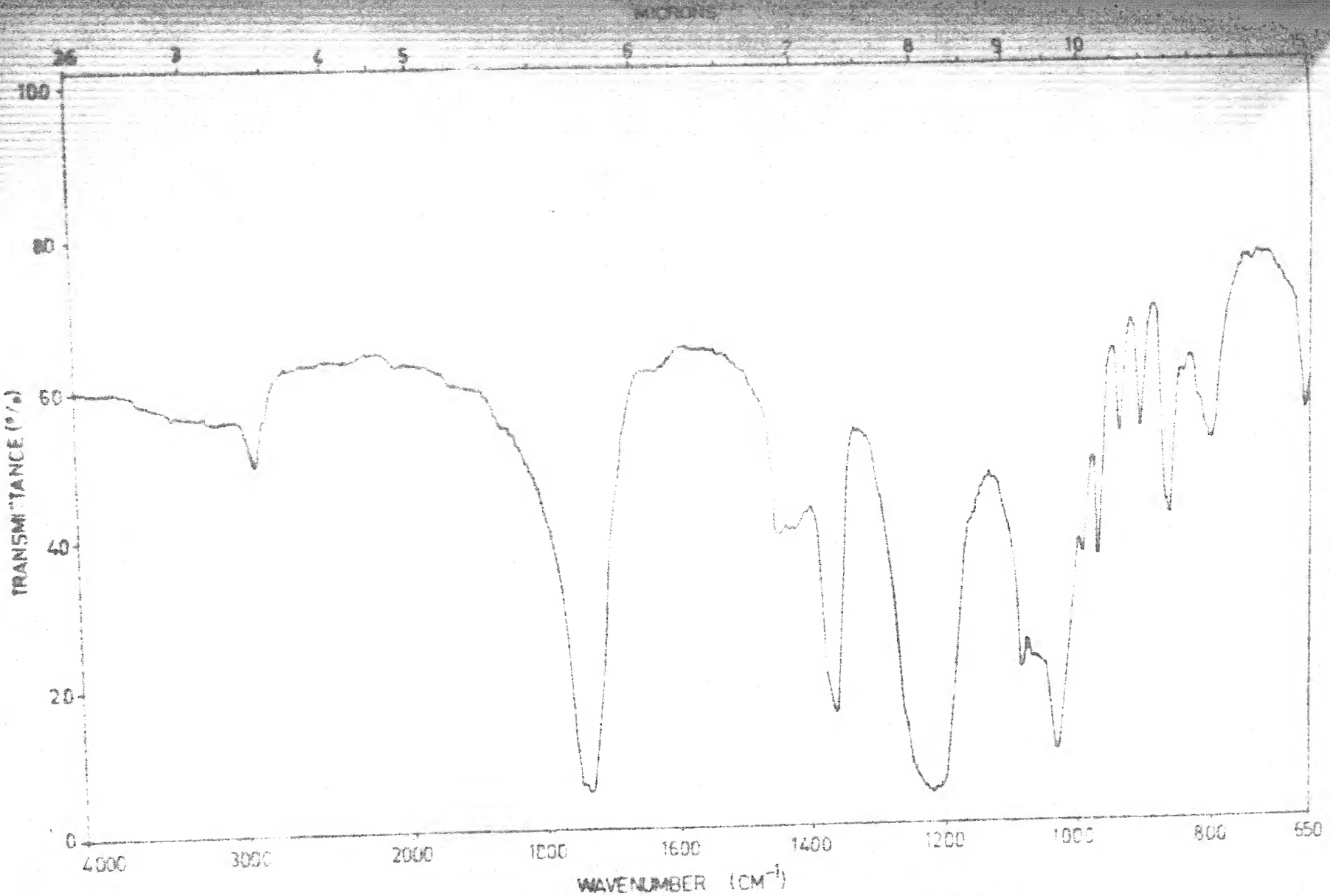


FIG-9 IR SPECTRUM OF SUBSTANCE-F

and the new bands at 1730 and 1220 cm^{-1} appeared to be a sugar alcohol. This was further supported by the NMR spectrum (Fig.10) of the acetate which exhibited four singlets at δ 1.96, 1.97, 1.99 and 2.1 representing the acetoxy signals. Two double doublets appeared at δ 4.01 and 4.11 (2H each, J=15 Hz and J=9 Hz) due to geminal coupling was attributed to C-1 and C-6 protons. The multiplets at δ 4.9 and δ 5.36 each integrating for two protons were due to C-2 and C-3 protons. The physico-chemical data of substance F and its acetate were found to be in conformity with the reported literature values of mannitol[109] and its acetate respectively. Finally its identity as mannitol (11) was established by co-paper chromatography in two different solvent systems[110-111] i.e. n-butanol acetic acid-water (4:1:5, v/v, upper, Rf=0.19) and ethyl acetate-acetic acid-water (3:1:3, v/v, Rf=0.19), mixed m.p. determination and super-imposable IR spectra with an authentic sample.

Substance G: It was obtained as yellow needles from methanol, m.p. 343-44° (lit. m.p. 347°)[112]. Elemental analysis and the molecular ion peak at m/z 270 in its mass spectra gave the molecular formula $C_{15}H_{10}O_5$. It gave a magenta colour with Mg/HCl (Shinoda test)[64] indicated it to be a flavone. The IR absorption (Fig.11) at 3380, 1656, 1620, 1586 cm^{-1} indicated the presence of hydroxyl chelated carbonyl group and aromatic nucleus with

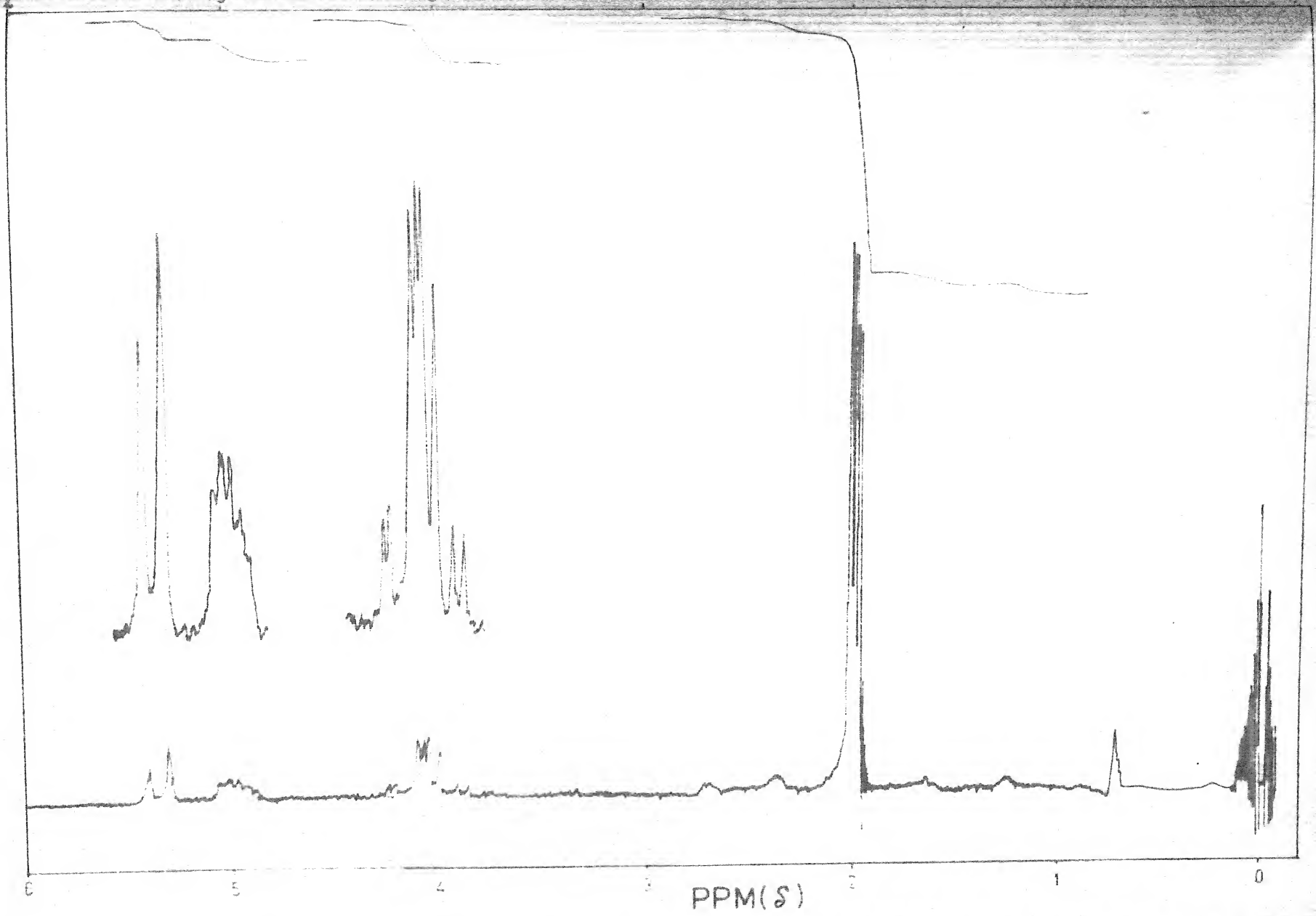


FIG-10 NMR SPECTRUM ACETATE OF SUBSTANCE-F

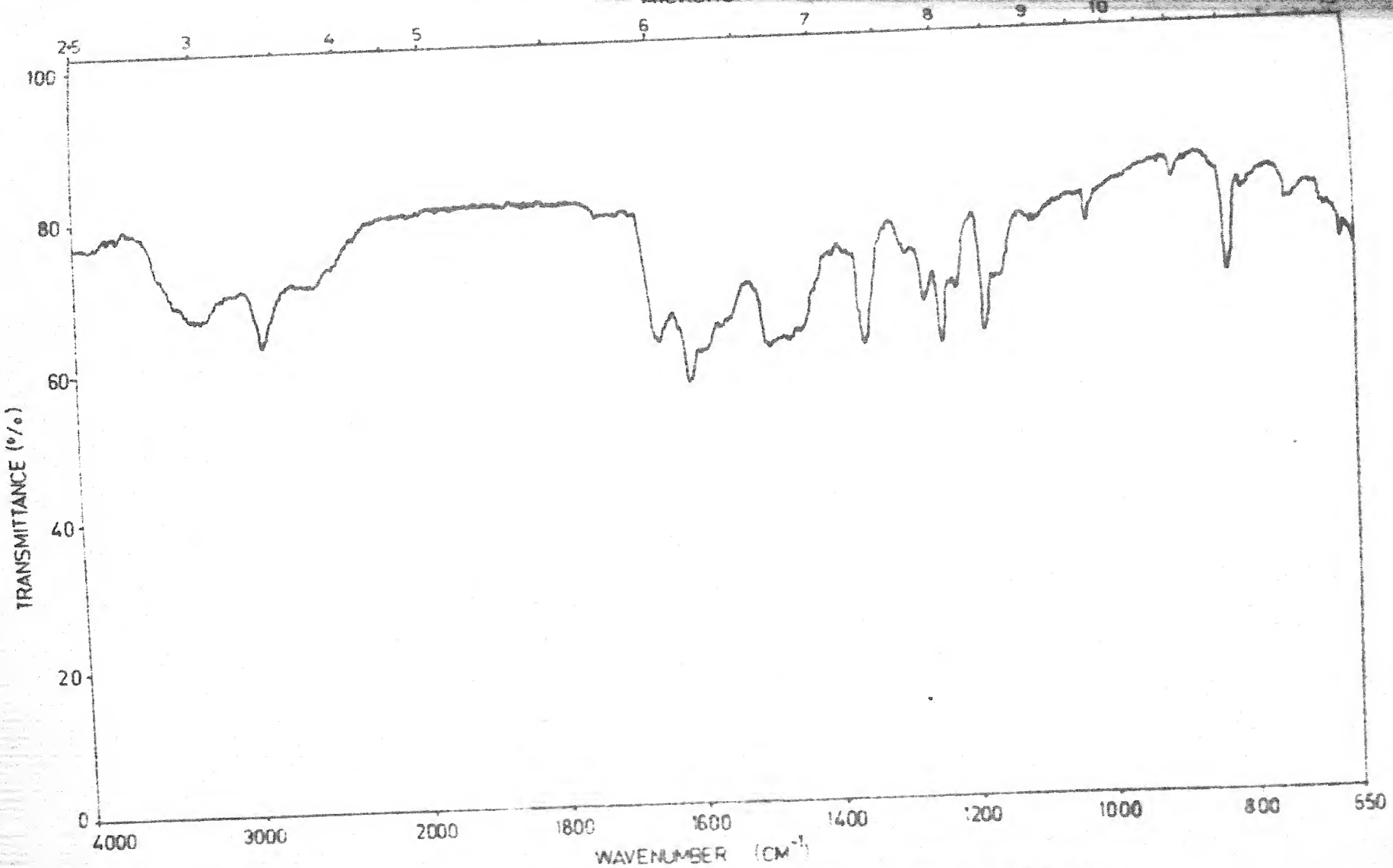


FIG-11 IR SPECTRUM OF SUBSTANCE-G.

a conjugated double bond. The stretching vibration at 1125 cm^{-1} indicated the presence of an ether linkage (Ar-O-C).

The UV spectrum (Fig.12) exhibited absorption maxima at 267, 296 and 336 nm. When aluminium chloride was added each of these absorptions split into two distinct peaks[113]. Band I underwent a bathochromic shift to give the pair of bands at 384 and 348 nm, whilst band II moved to 301 and 274 nm. There was no change in the λ max when a few drops of concentrated HCl was added to the above solution. The bathochromic shift of Band I (in MeOH) to Band Ia (in $AlCl_3$/HCl) is of 38 nm. The above data showed the absence of vicinal hydroxy group. The bathochromic shift with $AlCl_3$ could be attributed due to the presence of C-5 hydroxy group[114]. There was a bathochromic shift of about 7 nm in the λ max of Band II on addition of fused sodium acetate suggesting the presence of a free hydroxyl group at C-7. A 56 nm bathochromic shift in band I in presence of sodium methoxide was due to free hydroxyl group at C-4'.

The mass spectral fragmentation pattern[115] (Fig.13) of substance H showed RDA fragment ion at m/z 152 due to 5, 7-dihydroxylated ring A and another fragment ion at m/z 118 arising from 4' - hydroxylated ring B. Peaks were also observed at m/z 269, 242, 153, 124, 123 and 121 (Scheme-1). It formed a triacetate with C_5H_5N/Ac_2O, when crystallised from methanol it separated as colourless silky

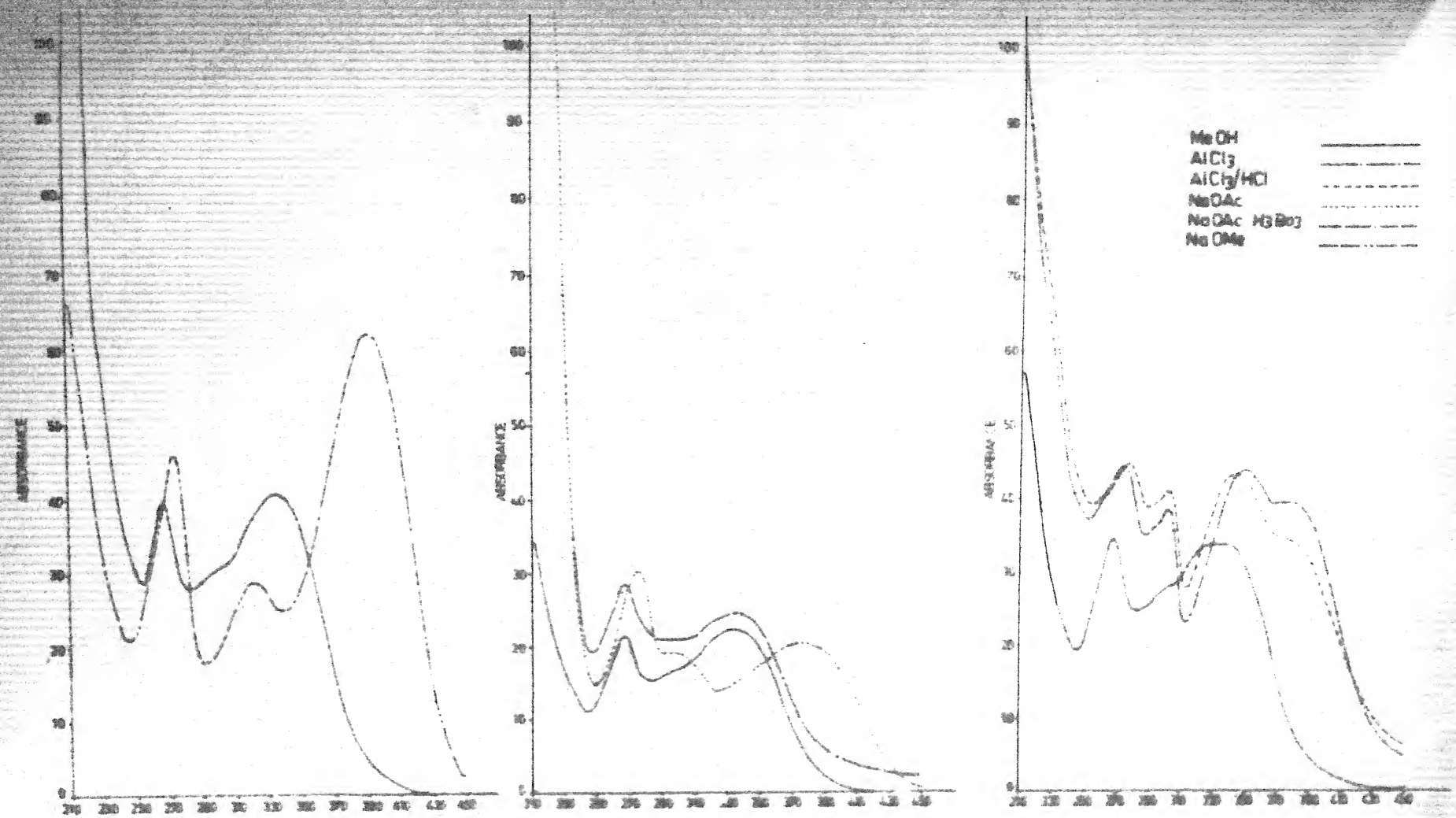


FIG-12 UV SPECTRUM OF SUBSTANCE-G

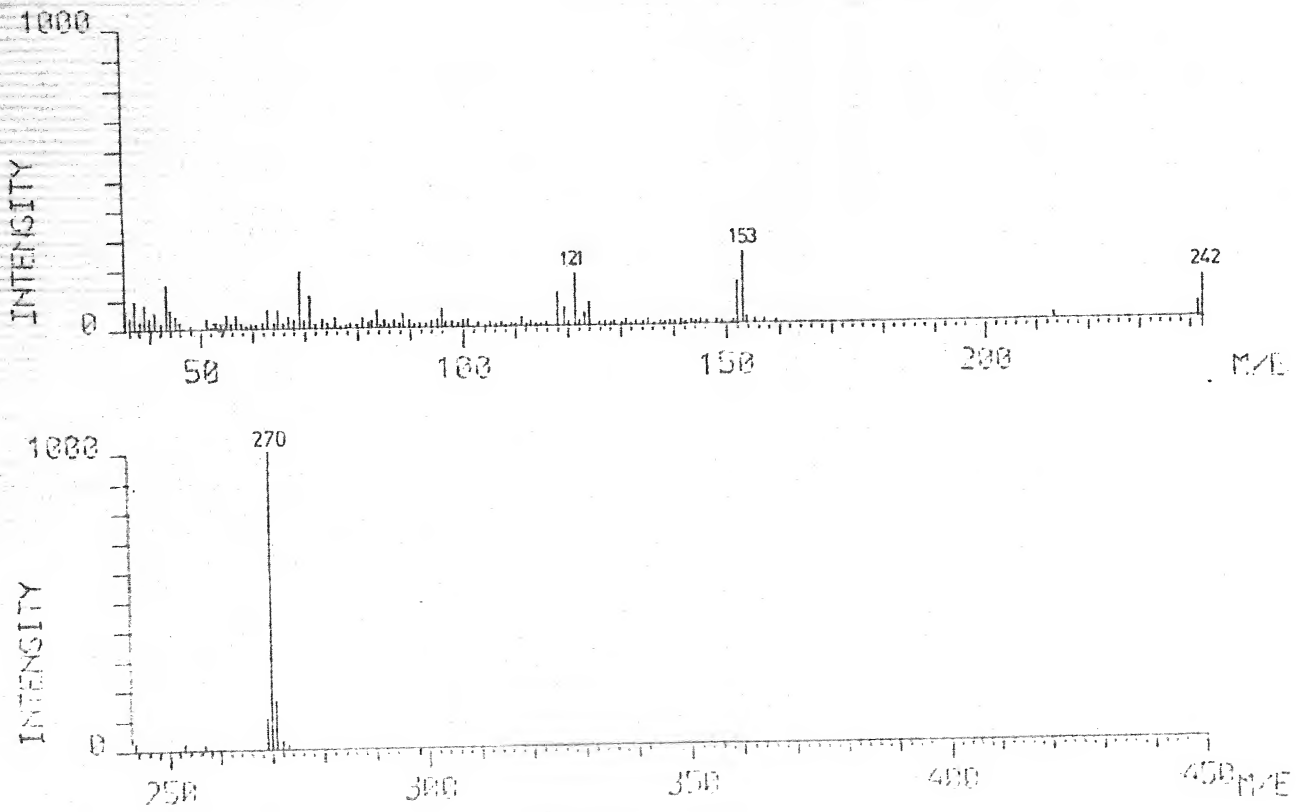


FIG-13 EI MASS SPECTRUM OF SUBSTANCE - G.

needles, m.p. 185-187°. Its IR spectrum exhibited an intense band due to acetyl carbonyl (1750 cm^{-1}) and was devoid of hydroxyl absorption. It was analysed for $C_{21}H_{16}O_8$ and was supported by mass spectrometry m/z 396(M^+). The above spectroscopic data together with a comparison with an authentic sample led to the characterisation of substance H as apigenin[116] (12).

Free sugars: The identity of free sugars were established by co-chromatography on Whatman paper No.1 with known reference samples using two different solvent systems[81-82] i.e.n-butanol-acetic acid-water (4:1:5, v/v, upper) and n-butanol-ethanol-water (4:1:5, v/v, upper) and aniline hydrogen phthalate[83] was used as detecting reagent. Glucose, arabinose and rhamnose were identified on the basis of identical Rf values with that of authentic samples.

STRUCTURE OF THE COMPOUNDS

$Me(CH_2)_{25}Me$ (1)

$Me(CH_2)_{26}Me$ (2)

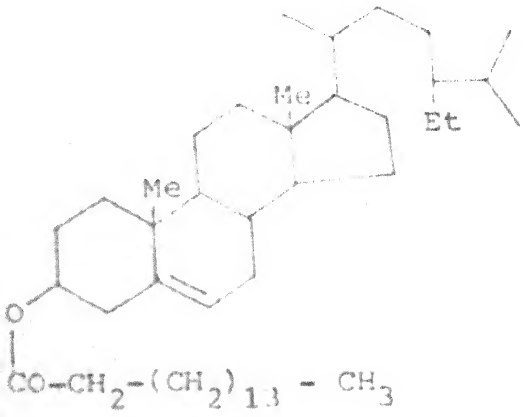
$Me(CH_2)_{27}Me$ (3)

$Me(CH_2)_{28}Me$ (4)

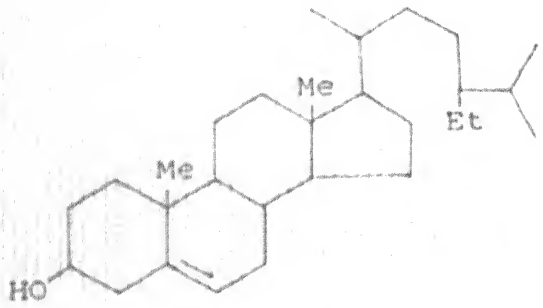
$Me(CH_2)_{29}Me$ (5)

$Me(CH_2)_{30}Me$ (6)

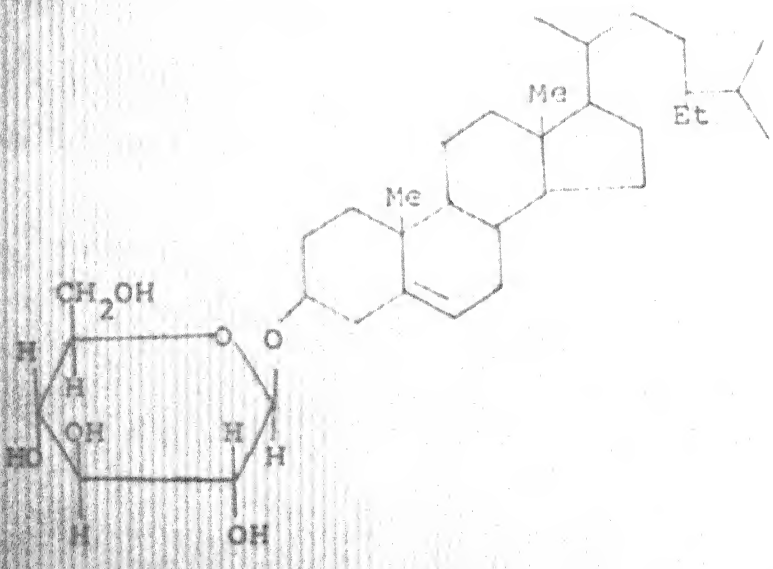
$Me(CH_2)_{31}Me$ (7)


Me
Et
Me
O
CO-CH2-(CH2)13 - CH3

(8)


Me
Et
Me
HO

(9)

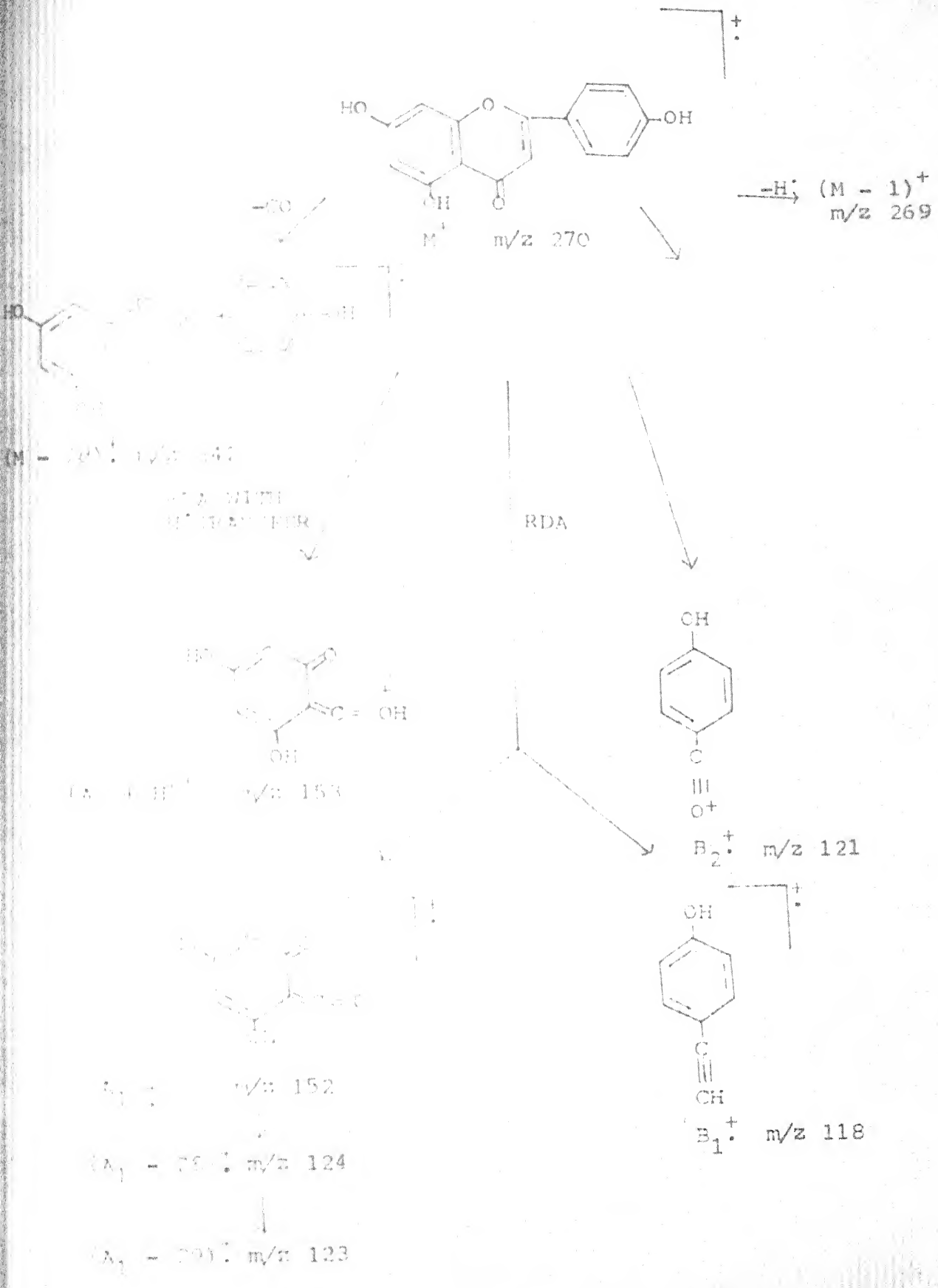

Me
Et
Me
CH2OH
O
H
H
HO
OH
H
H
H
OH

(10)

(11)

(12)

SCHEME - 1 : Mass Spectral fragmentation of Apigenin



EXPERIMENTAL

ISOLATION OF THE CONSTITUENTS:

The plant material was collected from the institute campus during monsoon season and was identified. It contained crude protein 13.5%, calcium 1.36% and phosphorus 0.18%. Shade-dried, powdered plant material (4 kg) was exhaustively extracted with ethanol (95%, 4x10 lit.) by cold percolation. The total ethanolic extract was distilled at 50° under reduced pressure. The concentrate on cooling deposited an inorganic crystalline material which was filtered off. It was identified as potassium nitrate (0.62%). The filtrate was concentrated and the extractive (350 g) was successively fractionated with hexane (4x2 lit.), benzene (4x500 ml) and acetone (4x1 lit). On concentration, the following fractions were obtained.

1. Hexane soluble (225 g).
2. Benzene soluble (3 g).
3. Acetone soluble (80 g).

COLUMN CHROMATOGRAPHY OF THE HEXANE FRACTION:

The dark green coloured hexane fraction was found to be a mixture of complex fatty materials. A part of it (80 g) was subjected to gross fractionation on a column of silica gel (500 g) in hexane. The elution was effected with hexane, benzene, ethyl acetate, acetone ethanol and mixtures of these solvents. The fractions (500 ml each)

were collected and combined on the basis of TLC and the results are summarised in Table-3.

Table-3 Column chromatography of hexane fraction (80 g).

Fr. No.	Eluant	Weight (g)	Remarks
1-4	Hexane	2.0	Contained substance A
5-10	Hexane	2.25	Contained substance B
11-13	Hexane:Benzene (90:10)	2.0	Complex oily mixture
14-17	Hexane:Benzene (70:30)	2.0	-do-
18-21	Hexane:Benzene (50:50)	3.0	-do-
22-26	Hexane:Benzene (30:70)	3.5	Contained substance C
27-34	Benzene	4.3	Complex mixture
35-39	Benzene:Ethyl acetate (10:90)	11.5	-do-
40-45	Benzene:Ethyl acetate (30:70)	6.5	-do-
46-49	Benzene:Ethyl acetate (50:50)	6.5	-do-
50-53	Benzene:Ethyl acetate (30:70)	7.0	Substance D
54-63	Ethyl acetate	10.0	Substance E
64-70	Acetone	3.0	Substance F
71-72	Acetone:Ethanol (70:30)	3.0	Mixture

SUBSTANCE A:

The fractions 1-4 were mixed and the solvent removed. The residue (2.0 g) was saponified with ethanolic potassium hydroxide (7%) and by usual work up yielded a colourless waxy product which was recrystallised from ethyl acetate (Substance A, 100 mg). M.p. 65-67°.

IR ν_{max}^{KBr} : 2920, 2850, 1460, 1380, 880, 740 and 720 cm^{-1}.

EIMS: m/z 464, 450, 436, 422, 408, 394, 380, 365, 351, 337, 323, 309, 295, 281, 267, 253, 239, 275, 211, 197, 183, 169, 155, 141, 127, 113, 99, 85, 71, 57 and 43.

FIMS: m/z 464 (M^+), 450 (M^+), 436 (M^+), 422 (M^+), 408(M^+), 394 (M^+) and 380 (M^+).

SUBSTANCE B: β-sitosterol palmitate

The fractions 5-10 were combined and after removal of the solvent gave the residue (2.25 g). Addition of acetone (10 ml) furnished a colourless solid (140 mg) which was found to be rich in substance B. PLC of this fraction on silica gel using benzene-methanol (9:1) as developing system afforded substance B (30 mg). Crystallised from hexane-benzene. M.p. 80-83°.

IR ν_{max}^{KBr}:	2920, 2870, 1750 (C=O), 1470, 1400, 1100, 900 and 720 cm^{-1}.
EIMS:	m/z 652, 638, 396, 255, 229, 215, 146, 111, 97, 85, 83, 71 and 57.
CIMS:	m/z 653 $(M+H)^+$, 639, 397, 383 and 257. m/z 670 $(M+NH_4)^+$, 397 and 274.
FDMS:	m/z 652 (M^+).

Alkaline hydrolysis of substance B:

Substance B (20 mg) and methanolic sodium hydroxide (1.0 ml, 25%) was refluxed on steam bath for 1 hr. It was cooled, then extracted with chloroform (5x2 ml) and washed free of alkali. The chloroform layer was separated, dried over anhydrous sodium sulphate and evaporated to afford β-sitosterol. It was crystallised from (chloroform-methanol). M.p. 136°. EIMS,m/z 414 (M^+). The aqueous layer on acidification and usual work up yielded palmitic acid. M.p. 62-63°. FDMS,m/z 252 (M^+).

SUBSTANCE C: β-sitosterol

The fractions 22-26 were mixed and concentrated to yield a residue (3.5 g). Addition of acetone (10 ml) gave a substance C (850 mg) which was recrystallised from

chloroform-methanol. The compound was identified as β-sitosterol on the basis of physical data, preparation of derivatives and comparison with known specimen (as reported).

SUBSTANCE D:

The fractions 50-53 were combined on the basis of TLC, the solvent evaporated off. The residue (7.0 g) was washed with ethyl acetate-benzene (70:30) mixture to yield a white solid of substance D (200 mg). M.p. 146°.

IR) ν_{max}^{KBr}: 3350, 3240, 2920, 2860, 1640, 1550, 1480, 1120, 1080, 1030, 900, 870 and 720 cm^{-1}.

FIMS: m/z 676 (M^+), 662 (M^+), 648 (M^+) and 620 (M^+).

SUBSTANCE E: β-sitosterol - β-D-glucoside

The fractions 54-63 were concentrated. The residue (10 g) was crystallised from methanol to afford substance E (850 mg).

The compound was identified as β-sitosterol-β-D-glucoside on the basis of physico-chemical data, preparation of derivative as reported in literature and comparison with the authentic sample.

SUBSTANCE F: Mannitol

The fractions 64-70 were combined and the solvent removed. The residue (3.0 g) was dissolved in ethanol (20 ml) and charcoaled to furnish white crystalline substance F (100 mg). Crystallised from ethanol. M.p. 166-67°.

IR/ $\overset{KBr}{max}$: 3420, 2940, 2880, 2820, 1460, 1375, 1320, 1260, 1200, 1085, 1020, 950, 930, 890, 740, 720 and 630 cm^{-1}.

EIMS: m/z 182(M^+), 165, 146, 133, 129, 115, 103, 85, 73, 61, 56 and 43.

FDMS: m/z 183 $(M+H)^+$.

Analysis: Found C, 39.50 ; H, 7.63

$C_6H_{14}O_6$ required C, 39.56 ; H, 7.69%

Acetylation of substance F:

Compound F (20 mg) was dissolved in acetic anhydride and dry pyridine (1 ml each). The reaction mixture was heated on a steam bath for 4 hrs. The solvents were removed by heating under vacuum. The residue was taken up in chloroform (4x10 ml). The chloroform layer was washed thoroughly with distilled water, dried over anhydrous sodium sulphate and the solvent removed. The

residue was crystallised from methanol to afford acetate (25 mg). M.p. 120-21°.

IR) ν_{max}^{KBr} : 1730, 1450, 1375, 1220, 1030, 920, 910, 865 and 800 cm^{-1}.

NMR($CDCl_3$): δ 1.96, 1.97(s, 3H, 2xOCOC$\underline{H}_3$), 1.99 and 2.1(s, 6H, 4xOCOC$\underline{H}_3$), 4.01 (J= 15 Hz, dd, 2H, C-1$\underline{H}$, 4.11 (J= 9 Hz, dd, 2H, C-6$\underline{H}$), 4.9 and 5.36 (m, 2H, C-5$\underline{H}$, C-2$\underline{H}$ and C-4$\underline{H}$, C-3$\underline{H}$).

EIMS: m/z 434 (M^+).

COLUMN CHROMATOGRAPHY OF THE ACETONE FRACTION:

The dark brown acetone soluble fraction was found to be a complex mixture on TLC. A part (70 g) of it was applied to a column of silica gel (450 g) in the form of slurry and eluted with benzene followed by ethyl acetate, acetone and ethanol in their increasing proportions. The fractions (250 ml each) were mixed on the basis of TLC and the results are summarised in Table-4.

Table-4 Column chromatography of acetone fraction (70 g).

Fr. No.	Eluant	Weight (g)	Remarks
1-4	Benzene	0.27	Mixture
5-15	Benzene:Ethyl acetate (80:20)	4.1	Contained substance G
16-21	Benzene:Ethyl acetate (60:40)	5.2	Complex mixture
22-34	Benzene:Ethyl acetate (40:60)	8.5	-do-
35-45	Benzene:Ethyl acetate (20:80)	3.5	Greenish material
46-55	Ethyl acetate	10.7	-do-
56-64	Ethyl acetate:Acetone (70:30)	11.0	Yellow complex mixture
65-72	Ethyl acetate:Acetone (50:50)	6.3	-do-
73-80	Acetone	7.0	-do-
81-83	Acetone:Ethanol (80:20)	2.0	Syrupy residue
84-86	Aqueous ethanol	1.0	Contained free sugars

SUBSTANCE G: Apigenin

The fractions 5-15 after removal of the solvent gave the residue (4.1 g) which was found to be rich in substance G. PLC of this fraction on silica gel using benzene-methanol (80:20) as developing system yielded substance G (50 mg). Crystallised from methanol. M.p.343-344°.

IR ν_{max}^{KBr} :		3330, 1656, 1620, 1586, 1500, 1440, 1350, 1270, 1240, 1180, 1125, 1040, 1020, 910, 840 and 740 cm^{-1}.
UV:	(MeOH) λmax	267, 296sh and 336 nm.
	(NaOMe)	275, 324 and 392 nm.
	($AlCl_3$)	276, 301, 348 and 384 nm.
	($AlCl_3$/HCl)	276, 299, 340 and 381 nm.
	(NaOAc)	274, 301 and 376 nm.
	($NaOAc/H_3BO_3$)	268, 302sh and 338 nm.
EIMS:		m/z 270(M^+), 269, 242, 153, 152, 124, 123, 121 and 118.
Analysis:		Found C, 66.4 ; H, 3.5
$C_{15}H_{10}O_5$		required C, 66.7 ; H, 3.7%

Acetylation of substance G:

Substance G (10 mg) was heated with dry pyridine (0.5 ml) and acetic anhydride (1 ml) for 4 hrs at 98°. The solvents were removed under vacuum. The residue was treated with water and extracted with ether (4x5 ml), dried (anhydrous sodium sulphate) and the solvents removed. The residue was crystallised from methanol as colourless needles (12 mg). M.p. 179-182°.

IR ν_{max}^{KBr} :	1750, 1635, 1600, 1220, 1200 and 840 cm^{-1}.

EIMS: m/z 396 (M^+).

Analysis: Found C, 63.9 ; H, 4.05

$C_{21}H_{16}O_3$ required C, 63.6 ; H, 4.0%

IDENTIFICATION OF FREE SUGARS:

The fractions 84-86 was concentrated under reduced pressure. It reduced Fehling's solution indicating the presence of free reducing sugars. The concentrate was applied on Whatman No.1 filter paper alongwith the known reference sugar samples in two different developing systems i.e. n-butanol-acetic acid-water (4:1:5, v/v,upper) and n-butanol-ethanol-water (4:1:5, v/v, upper). The spots were visualised by aniline hydrogen phthalate. This fraction was found to contain three spots having identical Rf values with that of glucose, rhamnose and arabinose.

REFERENCES

1. Gulland, J.M. and Robinson, R., J.Chem.Soc., 123, 980 (1923).

2. Woodward, R.B. and Doering, W.E., J.Amer.Chem.Soc., 66, 849 (1944).

3. Neuss, N., Boaz, H.E. and Forbes, F.W., J.Amer.Chem.Soc., 76, 2463 (1954).

4. Clemo, G.R., Haworth, R.D. and Walton, E., J.Chem.Soc., 1110 (1930).

5. Vyazmenskii, E.S., Pharmacology and Toxicology, 10 51 (1947).

6. Ettlinger, M.G. and Lundeen, A.J., J.Amer.Chem.Soc., 78, 4172 (1956).

7. Elderfield, R.C., Chem.Rev., 17, 184(1936).

8. Elderfield, R.C., J.Biol.Chem., 113, 631 (1936).

9. Bloch, R., Rangaswami, S. and Schindler, O., Helv.Chim.Acta., 43, 652 (1960).

10. Bentley, K.W., The Chemistry of Natural Products. Inter science Publishers, Inc., New York, Vol.4, P. 1, 226 (1960).

11. Chopra, R.N., Nayar, S.L. and Chopra, I.C., Glossary of Indian Medicinal Plants, C.S.I.R. New Delhi, P.9 (1956).

12. Koike, H., Folia.Pharmacol.Japan., 12, 89 (1931).

13. Fakuda, T., Arch.Exptl.Path.Pharmacol.,164, 685 (1932).

14. Clark, W.G. and Geissman, T.A., J.Pharmacol.Exper. Therap., 95, 363 (1949).

15. Collins, R.A., Schreiber, M. and Elvehjem, C.A., J.Nutrition, 49, 589 (1953).

16. Ambrose, A.M., Robins, D.J. and Deeds, F., Fed.Proc., 9, 264 (1950).

17. Sokoray, L. and Czimmer, A.G., Arch.Exptl.Path.Pharmacol., 190, 622 (1938).

18. Pearson, N.N., J.Am.Med.Assoc., 164, 1675 (1957).

19. Murthi, V.V.S., Rao, N.V.S. and Seshadri, T.R., Proc. Ind.Acad.Sci., 25A, 22 (1947).

20. Murthi, V.V.S., Row. L.R. and Seshadri, T.R., Proc.Ind. Acad. Sci., 27A, 33 (1948).

21. Murthi, V.V.S. and Seshadri, T.R., Proc.Ind.Acad.Sci., 27A, 258 (1948).

22. Seshadri, T.R. and Varadarajan, S., Proc.Ind.Acad.Sci., 35A, 75 (1952).

23. Seshadri, T.R. and Viswanadham, N. Proc.Ind.Acad.Sci., 25A, 337 (1947).

24. Chari, N.N. and Seshadri, T.R., Proc. Ind.Acad.Sci., 27A, 128 (1948)

25. Limaye, D.B., Abstr.Proc.Ind.Sci.Congress, 151 (1926).

26. Subramanian, S.S. and Nair, A.G.R., Indian.J.Chem.,4 , 461 (1966).

27. William, A.H., Chem.and Ind., 1318 (1964).

28. Wallis, E.S. and Chakravorty, P.N., J.Org.Chem., 2, 335 (1938).

29. Cook, J.W. and Paige, M.F.C., J.Chem.Soc.,336 (1944).

30. Anderson, R.J., Shriner, R.L. and Burr, O.G., J.Amer.Chem.Soc., 48, 2987 (1926).

31. Heilbron, I.M. Jones, E.R.H., Roberts, K.C. and Wilkinson, P.A., J.Chem.Soc., 344 (1941).

32. Steinhart, C.E., Anderson, L. and Skoog, F., Plant.Physiol., 37, 60(1962).

33. Bouwkamp, J.C. and Mcardle, R.N., Hort.Science, 15, 69 (1980); Chem.Abstr., 92 158890 (1980).

34. Biol.Abstr. 23, 29938 (1949).

35. Cooks, R.G. and Johnson, G.S., In Mass Spectrometry Specialist Periodical Reports, The Chemical Society, London, Vol.1 (1971).

36. Schlunegger, U.P., Topics in Current Chemistry, Springer Verlag, Vol. 95, P.49 (1981).

37. Munson, B., Anal.Chem., 49, 722A (1977).

38. Wood, G.M., Tetrahedron, 38, 1125 (1982).

39. Martinelli, E.M., Europ. J.Mass Spectrum, 1 , 33 (1980).

40. Geiger, H. and Schwinger, G., Phytochemistry, 19, 897 (1980).

41. Budzikiewicz, H., Djerassi, C. and Williams, D.H., Mass Spectrometry of Organic Compounds, Holden-Day, San Francisco, Vol. 2, (1967).

42. Lang, L., Absorption Spectra In The Ultraviolet and Visible Region, Publishing House of the Hungarian Academy of Sciences, Budapest, P.1 (1961).

43. Dyer, J.P., Application of Absorption Spectroscopy of Organic Compounds, Prentice Hall of India, New Delhi, P.28 (1978).

44. Berl, W.G., Physical Methods in Chemical Analysis, Academic Press, P.1 (1956).

45. Dilts, R.V., Analytical Chemistry, Methods of Separation, D.Van. Nostrand Company, New York, P.503 (1974).

46. Pryde, A. and Gilbert, M.T., Application of High Performance Liquid Chromatography, Chapman and Hall London, P.1 (1979).

47. Wealth of India, (Raw Material) C.S.I.R.,New Delhi Vol.1, P.66, (1948).

48. Kirtikar, K.R. and Basu, B.D., Indian Medicinal Plants, Basu, L.M. Allahabad, Vol.1, P.752 (1935).

49. Chopra, R.N., Nayar, S.L. and Chopra, I.C., Glossary of Indian Medicinal Plants, C.S.I.R., New Delhi, P.15 (1956).

50. French , R.B. and Abbott, C.D. Agr.Expt.Sta.Tech.Bull., 444, 21 (1948); Chem.Abstr., 43, 3536 (1949).

51. Rai, P.and Kanodia, K.C., Indian J.Ecol., 7, 268 (1980); Chem.Abstr., 94, 138096 (1981).

52. Dyer, J.R., Application of Absorption Spectroscopy to Organic Compounds, Prentice-Hall of India,New Delhi, P.38 (1978).

53. Carnduff, J., Hargreaves, K.R. and Nechvatal, A., Phytochemistry, 5, 1029 (1966).

54. Hildebrand, J.H. and Wachter, A., J.Amer.Chem.Soc. 51, 2487 (1929).

55. Bell, K.H. and Duewell, H., Aust.J.Chem. 662 (1961).

56. Chibnali, A.C., Piper, S.H., Pollard, A., Williams, E.F. and Sahai, P.N., Biochem.J., 28, 2189 (1934).

57. White, J.W. and Santi, F.R., J.Amer.Chem.Soc. 67, 881 (1945).

58. Betrabet, M.V. and Chakravarti, G.C., J.India.Inst.Sci., 16A, 41 (1933).

59. Robinson, G.M., J.Chem.Soc., 1534 (1934).

60. Jain, R. and Gupta, R.K., J.Indian Chem.Soc.,58, 819 (1981).

61. Steiner, M. and Holtzen, H., Modern Methods of Plant Analysis, ed. Peach, K. and Tracey, M.V., Springer-Verlag, Berlin, Vol.3, P. 64 (1955).

62. Fieser, L.F. and Fieser, M., Reagents for Organic Synthesis, John Wiley and Sons. Inc.New York, P.1147 (1966).

63. Anderson, R.J., Shriner, R.I. and Burr, O.G., J.Amer.Chem.Soc., 48, 2987(1926).

64. Geissman. T.A., Modern Methods of Plant Analysis, ed, Peach. K. and Tracey, M.V., Springer-Verlag, Berlin, Vol.3, P.471 (1955).

65. Feigl, F., Spot-Tests Organic Application, Elsevier Publishing Company, London, P.289 (1954).

66. Mabry, T.J, Markham, K.R. and Thomas, M.B., Systematic Identification of Flavonoids., Springer-Verlag, New York P.53 (1970).

67. Hasegawa, H. and Shirato, T., J.Amer.Chem.Soc., 74, 6114 (1952).

68. Veno, A., J.Pharm.Soc.Japan., 82, 1479 (1962).

69. Mabry, T.J.Morkham, K.R. and Thomas, M.B.Systematic Identification of Flavonoids, Springer-Verlag,New York Chapter 5, Spectra No.69, 65, 66, 40, 58 (1970).

70. Clark, H.I. and Simon, H.W., J.Amer.Chem.Soc., 75, 50 (1953).

71. Hattori, S., The Chemistry of Flavonoid Compounds, ed. Geissman, T.A., Pergamon Press, New York, P.338 (1962).

72. Gripenberg, J., The Chemistry of Flavonoid Compounds, ed, Geissman T.A., Pergamon Press,New York, P.437(1962).

73. Smirnova, L.P., Glyzin, V.I., Patudin, A.V. and Bankovskii, A.I., Khim.Prir.Soedin., 668 (1974); Chem.Abstr., 82, 83016 (1975).

74. Karrer, W. Constitution and Vorkommen der Organischen. Pflanzenstoffe, Birkhauser Basle, P.117 (1958).

75. Vogel, A.I., Qualitative Chemical Analysis. Longmans, Green and Co., London, P.545 (1948).

76. Postemak, T., Reymond, D and Haerdi, W.Helv.Chim.Acta. 38, 191 (1955).

77. Nilsson, M., Acta.Chem.Scand., 10, 413 (1956).

78. King, F.E., Lewis, J.W.C. and Forbes, W.F., J.Chem.Soc., 2948 (1955).

79. Mabry, T.J., Markham, K.R. and Thomas, M.B. Systematic Identification of Flavonoids, Springer-Verlag,New York P.269 (1970).

80. Stepien. O., Pr.Nauk.Akad.Med.Wrodawia, Poland, 71, 41 (1973)., Chem.Abstr., 82, 13949 (1975).

81. Patridge, S.M., Biochem.J., 42, 238 (1948).

82. Bradfield, A.E. and Flood, A.E., Nature, 166, 264(1950).

83. Patridge, S.M. Nature, 164. 443 (1949).

84. Official Methods of Analysis of the Association of official Agricultural Chemists., ed. Horowitz, W., A.O. A.C. Washington D.C., 9th edition, P.94 and 369 (1960).

85. Hooker, J.D., Flora of British India. Reeve, L. and Co. Ltd., Kent, England, Vol.2, P. 158 (1887).

86. Easterfield, T.H. and Taylor, C.M., J.Chem.Soc., 99, 2305 (1911).

87. Chibnali. A.C., Piper, S.H., Mangouri, H.A.E., Williams, E.F. and Iyengar, A.V.V., Biochem.J., 31, 1981 (1937).

88. Ageta, H., Yakugaku Zasshi, 79, 58 (1959); Chem.Abstr. 53, 10032 (1959).

89. Robinson, G.M., J.Chem.Soc., 543 (1934).

90. Savidan, L., Ann.Chim., 1, 53 (1956); Chem.Abstr., 52, 19185 (1958).

91. Fritz, M.B., Bull.Soc.Chim.France, 516 (1957); Chem.Abstr., 51, 11071 (1957).

92. Chibnali, A.C., Piper, S.H., Pollard, A., Williams,E.F. and Sahai, P.N., Biochem.J., 28, 2189 (1934).

93. Lopez, J.B.B. and Marques, I.R. An.Real.Soc.Espan. Fis.Quim., 62, 871 (1966); Chem.Abstr., 66, 66939 (1967).

94. Swift, L.J. J.Amer.Chem.Soc., 74, 1099 (1952).

95. Kind, C.A. and Celentano, V.D., J.Org.Chem., 18, 1473 (1953).

96. Fletcher, H.G. and Findlay, G.R., J.Amer.Chem.Soc., 70, 4050 (1948).

97. Brownstein, S., J.Amer.Chem.Soc., 81, 1606 (1959).

98. Behari, M. and Andhiwal, C.K., Ind.J.Chem., 13, 639 (1975).

99. Tandon. S.P., Tiwari, K.P. and Saxena, V.K., Proc. Natn.Acad.Sci., India, 40, 217 (1970).

100. Slotta, K.H. and Primosigh, J., Nature, 168,696 (1951).

101. Wiggins, L.F. and Williams, J.H. Nature, 170, 279 (1952).

102. Toennies. G. and Kolb, J.J.Anal.Chem., 23, 823 (1951).

103. Chopra, R.N., Nayar, S.L. and Chopra, I.C., Glossary of Indian Medicinal Plants, C.S.I.R.,New Delhi, P.154 (1956).

104. Kirtikar, K.R. and Basu, B.D., Indian Medicinal Plants. Basu, L.M. Allahabad, Vol.3. P.1810 (1935).

105. Bhakuni, D.S., Dhar, M.L., Dhawan, B.N., Gupta, B. and Srimali, R.C. J.Exper.Biol. 9, 97 (1971).

106. Tiwari, K.P. and Choudhary, K.P., Phytochemistry, 18, 2044 (1977).

107. Tiwari, K.P. and Choudhary, R.N. J.Indian Chem.Soc., 57, 453 (1980).

108. King, F.E. and Jurd, L., J.Chem.Soc.,1192 (1953).

109. Wolfrom, M.L., Moody, F.B., Konigsberg and Goepp, R.M. J.Amer.Chem.Soc., 68, 578 (1946).

110. Buchanan, J.O. Dekker, C.A. and Long A.G., J.Chem.Soc., 3162 (1950).

111. Hackman, R.H. and Trikojus, V.M., Biochem.J., 51, 653 (1952).

112. Dictionary of Organic Compounds, ed. Heilbron, I., Cook, A.H., Bunbury, H.M., Eyre and Spottiswoode, London. P.264 (1965).

113. Mabry, T.J. Markham, K.R. and Thomas, M.B., The Systematic Identification of Flavonoids, Springer-Verlag, New York., P.53. (1970).

114. Jurd, L. and Geissman, T.A., J.Org.Chem., 21, 1395 (1956).

115. The Flavonoids, ed. Harborne, J.B., Mabry, T.J., and Mabry, H., Chapman and Hall, London, Chapter 3, P.82 (1975).

116. Sinha, N.K., Seth, K.K., Pandey, V.B., Dasgupta, B. and Shah, A.H., Planta Medica, 42, 297 (1981).

APPENDIX

NOTES

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Chemical Investigation of *Alysicarpus longifolius* Leaves

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ALYSICARPUS longifolius[1] Wright and Arn (Leguminosae), an annual herb commonly grows throughout the tropical region. The plant is reputed for its medicinal values[2] and potential use as a forage. Literature revealed that no work has been reported on the chemistry of this plant. Isolation and identification of myricyl alcohol, β-sitosterol, β-sitosterol acetate, rutin, pinitol along with an aliphatic ester and a flavonol are reported here. The latter two are being investigated further. The identity of the compounds was established on the basis of m.m.p. co-tlc, uv, ir, mass and comparison with authentic samples.

Experimental

The leaves were collected locally during July and August and contained crude protein 20.7, calcium 2.24 and phosphorus 0.29%. Powdered air-dried leaves (1 kg) were exhaustively extracted with ethanol (95%) under reflux. The alcoholic extract on concentration and cooling deposited a yellow crystalline compound (B). The remaining extract was successively fractionated into petroleum ether, benzene, ethyl-acetate and butanol fractions.

Petroleum ether fraction : It was chromatographed on silica gel column. Elution with different solvents and their mixtures yielded the following compounds. Compound A, (Petroleum ether-benzene 8 : 2). On crystallisation from acetone a white crystalline product m.p. 74°, MS (m/e M^+ 732) was obtained showing strong ir absorption at 1740 and 1175 cm^{-1} suggestive of an aliphatic ester. Myricyl alcohol[3] ; m.p. and mm.p. with an authentic sample, 86-87°, acetate, m.p. 70-71° ; β-sitosterol[3,4]- m.p. 136-137°, $(\alpha)_D - 33°$: acetate, m.p. 129° ; β-sitosterol acetate, m.p. 129°.

Ethyl acetate fraction : Rutin m.p. 186-187°, identical (m.p. and ir) with an authentic sample.

Butanol fraction : Pinitol m.p. 186°, identical (ms and ir) with an authentic specimen, penta-acetate, m.p. 98°.

Compound B, was obtained from alcohol as yellow crystals m.p. 197°, gave positive Shinoda Test[4-5] (Mg+HCl) for flavonol, uv $\lambda_{max}^{(MeOH)}$ 272, 335, nm : $\lambda_{max}^{(MeOH+AlCl_3)}$ 279, 352, nm : $\lambda_{max}^{(MeOH+AlCl_3+HCl)}$ 291, 352 nm : $\lambda_{max}^{(MeOH+NaOMe)}$ 277, 362 nm. There was no shift on addition of sodium acetate. The ir spectrum exhibited characteristic peaks at ν_{max}^{KBr} 3400, 2920, 1645, 1590, 1555, 1485 and 1445 ; acetate (acetic anhydride+pyridine) crystallized from methanol as colourless silky needles m.p. 179°. IR ν_{max}^{KBr} 1740 cm^{-1}, ms (m/e M^+ 656).

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References

1. The Wealth of India, "Raw Meterial", C. S. I. R. New Delhi. Vol 1, 1948, p. 66.
2. K. R. KIRTIKAR and B. D. BASU, "Indian Medicinal Plants", Vol. I, 2nd edition, p. 752.
3. I. HEILBORN, H. M. BUNBURY, A. H. COOK and E. R. H. JONES, "Dictionary of Organic Compounds", (Eyre Spotwood, London), 1965, Vol. 5, pp. 2902, 3093.
4. K. PEACH and M. V. TRACEY, "Modern Methods of Plant Analysis", (Springer Verlag, Berlin) 1955, Vol. 3A, pp. 167, 471.
5. T. J. MABRY, K. R. MARKHAN and N. B. THOMAS, "The Systematic Identification of Flavonoids", 1970, p. 41.
6. K. A. ZIRVI, K. JEWERS and M. J. NAGLER, "Phytochemical Investigation of *Prosopis glandulosa* stem", *Planta Medica*, Nov. 1977, p. 240.

APPENDIX